SYSTEM DISEASES OF BONE

Discussion Leader:

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NICHOLS: There are three reasons for including a discussion on "System Diseases of Bone" in a symposium of this type.

The first reason is that clinical bone disease occurs or will occur in the majority of us by the time we leave this planet. Osteoporosis, for example, can be shown to be present—even though it may be asymptomatic—in close to 50 percent of all women over 60 and is almost equally common in men, although it appears rather late in life. Since the origins and treatment of these diseases are largely unknown, there are good reasons for attacking these problems from a purely medical point of view. The clinicians badly need the help of their colleagues in basic science in finding the new answers which they seek.

A second reason is that this discussion is of use to those of us who are basic scientists if it brings to our attention the natural experiment provided by disease. Such experiments have told us more than once what questions to ask. Sometimes we have found the answer by turning to man, sometimes by turning back to the animal with new insight. Later I will present some examples which will perhaps illustrate this point.

Third, there are reasons, well illustrated by the discussions of earlier sessions, to think that we are at the beginning of a new era in our understanding of bone and its diseases. Those of us who are physicians will remember that bone disease was first described in terms of gross structural disturbance. This began, probably, as far back as the Egyptians and has gone on ever since. More recently, bone disease was studied in terms of mineral metabolism because it was easier to correlate the nutritional aspects of mineral metabolism with bone disease than anything else that could be measured at that time. So we went through an era dominated by Albright, in which external balances of calcium, phosphorus, and nitrogen were cor-

related with various forms of bone disease. We are now reaching a point where we can start to think about bone disease in yet another way, in terms of cell or tissue biology; it is to this aspect that I will address my remarks.

Bone disease, approached at the level of cell biology, can be thought about in quite simple terms. There are actually only two sets of processes going on: accretion and resorption. Once the details of these processes are known, including their initiation and control, it is possible to describe the phenomena of embryogenesis, of growth or remodeling, and perhaps of calcium and phosphorus homeostasis in terms of these two systems. By the same token, we will be able to describe bone disease in terms of the derangement of these two processes and the balance between them.

I will speak almost entirely about matrix, partly because one can deal with only one thing at a time and partly because matrix appears as the primary material which determines form; the chemical characteristics of the matrix, once they have been laid down, serve to attract the necessary mineral to provide rigidity. Although mineral metabolism may affect the processes of resorption and secretion of matrix in some situations, in general it tends to be a follower rather than a leader.

The first step in developing these ideas is to list those things we wish to know in order to look at bone disease. We need to understand the nature and the sequence of the steps involved in biosynthesis of matrix, the substrate requirements and rate of each. Similarly, we must know how resorption takes place and what steps in the process are rate limiting. Finally, we will need to know what controls exist for each overall process and how they work. This category includes not only such things as substrate availability and hormones but also a host of other factors, which may well be the most important of all, such as responses to local stimuli. This information will provide answers to such intriguing questions as "What tells bone cells that a fracture has occurred and callus formation is needed?"

Figure 85, which I prepared for another symposium a year ago, summarizes the state of our knowledge about bone-cell metabolic systems and provides us with ideas of where controls might be applied. The information on which this diagram is based has been provided by a number of sources, some dealing with bone and some with other connective tissues. As can be seen, we have quite a bit of biochemical information about bone cells. There are transport systems for glucose and amino acids (shown by the circle at the left of the cell) which get the raw materials in. We know that amino acids are concentrated in the cell either by a transport system or by synthesis from glucose as a precursor. It has also been shown that these amino acids are activated,

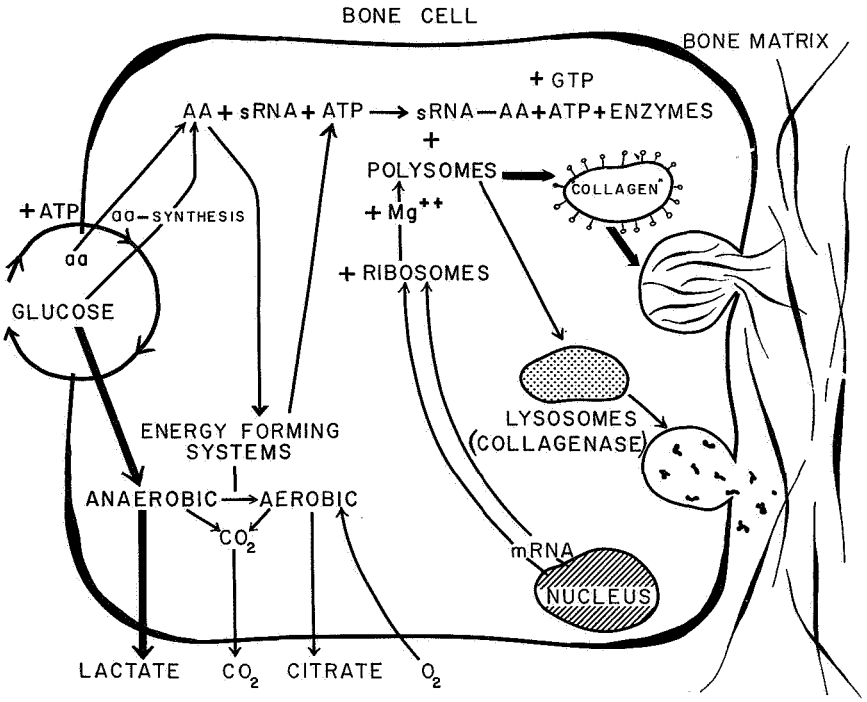

FIGURE 85. Bone-cell metabolic systems.

tied onto their appropriate transfer RNA's, and assembled, presumably by a ribosomal mechanism, into collagen which is then stored in vesicles and finally extruded into the extracellular phase. The rate of each of these steps and of the overall process can be measured. A second arrow emerging from the protein synthetic scheme, and leading to lysosomes and collagenase, has been included to remind us that these factors are present and involved in resorption. These collagenase-containing bodies have been shown as storage vesicles which also extrude their contents extracellularly to indicate that the collagenase must work outside rather than inside the cell. Whether two separate ribosomal paths exist (in the same or different cells) for these two processes is not known, but the presumption can be made that the information for the biosynthesis of each flows from the nucleus via messenger RNA.

Energy to drive these systems is needed; so ATP is formed by a breakdown of glucose, partly by glycolysis and production of lactic acid and partly by an oxygen utilizing system with the production of CO_2 and, in this particular cell, the release of citric acid as well. The relation of this acid production to bone-mineral mobilization and hence resorption has been suggested so often that it need not be reviewed here.

One of the conveniences of this scheme is that it permits potential sites for control of rates of accretion and resorption to be seen at a glance. For example, the deposition of extracellular collagen could be controlled by such varied factors as change in transmission of nuclear information, availability of energy, availability of substrate, variations in the biosynthesis of amino acids from glucose, or release of collagen from storage vacuoles. Similarly, resorption rate is clearly dependent on rates of acid production, biosynthesis of enzymes, and the mechanisms for delivery of enzymes into the extracellular space.

These thoughts, in turn, lead to a very simple classification for various bone diseases (table XIV). There are just three kinds of disturbances that can occur: (1) a defect in cellular machinery, (2) a deficiency in the availability of raw materials, or (3) a disturbance in the controls that regulate the rates of one or more steps; the latter disturbance can be subdivided into three categories: (*a*) hormonal, (*b*) local, and (*c*) "informational." This scheme with examples of diseases that, according to present information, fit into each category are shown in table XIV. Osteogenesis imperfecta, in which collagen synthesis seems to be deficient or deranged, appears to be a good example of a defect in cell machinery. Nutritional deficiency has been illustrated by osteomalacia, but scurvy could be cited equally well. The hormonal example is obvious, but perhaps the choices for the "local" and "informational" categories need some defense. Osteoporosis was picked for the former category because there are aberrations of the metabolic pattern in the bone cells in such patients, but so far no cause outside of the cells has been found for these changes. Another example which might be even better would be "fracture." It was omitted because it seemed a local rather than a systemic bone disease. Myeloma has been used because it is a widespread diffuse tumor that often produces bone disease that, if our recent evidence can be believed, may well result from a redirection of cellular biosynthetic activity.

TABLE XIV

CLASSES OF BONE DISEASE

Type	Example
(1) Defects of cellular machinery	Osteogenesis imperfecta
(2) Deficiency of substrates	Osteomalacia
(3) Disturbance of controls:	
(*a*) Hormonal	Hyperparathyroidism
(*b*) Local	Osteoporosis of disuse
(*c*) "Informational"	Multiple myeloma

RAISZ: Why not put myeloma under (2)?

NICHOLS: Because we have some experimental data which suggest that it should not be.

BUDY: What about genetic defects? Do you put them all under cellular machinery defects?

NICHOLS: Yes, I have, Dr. Budy, on the basis that most genetic defects eventually turn out to be enzyme deficiencies.

FREMONT-SMITH: That is information, is it not?

NICHOLS: I put it where I did because I have recently obtained some experimental information suggesting that genetic defects are probably not nutritional.

PRITCHARD: Where would an osteogenic sarcoma come into the system?

NICHOLS: I am not sure, but at present I would put it with myeloma.

PRITCHARD: What about infection with viruses and bacteria?

NICHOLS: I do not know. Where should I put them?

PRITCHARD: In nutrition?

URIST: Make a special category for infection.

FREMONT-SMITH: And have another one for miscellaneous.

NICHOLS: One must have a scheme in mind to guide one's work, and this is the scheme under which we have studied 103 patients with all sorts of diseases. In some instances, there are enough patients and the results are consistent enough for a pattern to be assigned to a disease; for example, hyperparathyroidism. Since we have already published these findings (ref. 152), I will not dwell on them. Instead, I would like to present some data on osteoporosis, in the hope that you will be able to help me understand what they mean; then I would like to go on to the skeletal response to fluoride. We will show an animal model, as well as some human data. Dr. Peck also has some information which I hope he will bring out (ref. 153) and thus move the discussion to the changes induced by multiple myeloma.

Listed in table XV are bone metabolic data for 17 patients with osteoporosis and who range in age from 39 to 78 years (ref. 154).

Three patients were treated with sodium fluoride for 1 year prior to biopsy, and one was studied before and after 1 year of sodium fluoride treatment. The metabolic data included in this table were derived from *in vitro* studies of biopsy samples and include measurements of several aspects of bone-cell function summarized in our hypothetical cell. The rate of collagen synthesis was measured in two ways: (1) from the incorporation of labeled proline, which reflects synthesis from preformed amino acids, and (2) from glucose, which measures amino acid as well as protein synthesis. Accumulation of label from these substrates in the cell fraction reflects a variety of cellular ac-

TABLE XV.—METABOLIC DATA FOR SOME OSTEOPOROSIS PATIENTS [a]

Patient	Sex	Age	DNA/100 mg wet weight	O_2 uptake, μmoles/hr/mg DNA	Lactate, μmoles/hr-mg DNA	Proline, μmoles/hr/mg DNA		Glucose, μmoles/hr/mg DNA	
						Cells	Collagen	Cells	Collagen
H–21	F	64	0.198	0.48	0.59	30.7	0.60	.....	.....
H–24	F	48	.198	1.05	1.09	22.9	1.29	.....	.....
H–29	F	78	.107	.65	1.40	12.6	.87	.....	.....
H–30a	F	57	.137	.72	1.16	39.0	.47	.....	.....
H–30b	F	58	.054	.78	.74	57.6	.28	1321.2	5.14
H–32	F	67	.241	.00	.92	53.2	.06	556.5	.43
H–34	M	68	.167	.56	.74	22.6	.13	.....	.....
H–35	F	47	.161	.91	1.39	46.3	.33	192.6	5.23
H–38	M	61	.140	.71	.80	26.8	.08	156.6	3.19
H–40	M	42	.187	.98	.64	11.4	.13	179.8	2.90
H–42	M	39	.121	.00	.40	56.5	.12	392.1	1.27
H–44	F	65	.126	.00	.80	15.2	.13	262.9	4.92
H–46	F	64	.162	.10	.81	101.0	.17	1167.0	5.49
H–48	F	71	.084	.00	.90	19.3	.16	182.9	2.56
H–49	F	53	.150	.54	.92	38.7	.16	311.6	5.53
H–50	F	44	.187	.41	.72	44.3	.12	950.9	7.66
H–53	M	57	.108	.50	.89	50.6	.25	445.6	6.44
H–71	M	62	.104	1.05	.85	33.0	.21	442.8	16.10
Normals: [b]									
Mean	.....	.....	.188	.47	.79	23.4	.24	320.0	4.00
S.D.	.....	.....	.290	.22	.29	7.84	.09	170.0	.92
Number of samples	.....	.....	6	8	6	5	5	4	5

[a] It should be noted that errors in the values for the standard deviations of the values in normals presented in the original publication (ref. 154) have been corrected here. [b] Age of normal subjects: 17–43 years; sex of normal subjects: 5 males, 3 females.

tivities. We now know (ref. 155) that proline label is to be found concentrated in the cell sap as the free amino acid, as well as incorporated into collagen and constituent cellular proteins. While some proline label is probably present in metabolic intermediates as well, much more of the glucose label is to be found in such materials, as Cohn and Forscher (ref. 156) first showed. We also know that glucose label must be present in free amino acid (ref. 157), in nascent collagen, and in cell proteins as well. Thus, glucose label in cells reflects many functions and probably should be taken as an index of overall metabolic activity. Finally, energy metabolism is estimated in two ways: (1) by O_2 uptake and (2) by lactate production, the latter perhaps being related to bone-mineral resorption as well.

At the bottom of the table, the means of values derived from eight normal subjects together with their standard deviations are shown for comparison. While bone from eight normal subjects was studied, data in some categories were collected from as few as four. The combination of the small number of subjects and of the considerable variability in metabolic activity that seems to occur from sample to sample resulted in standard deviations as large as 50 percent of the mean. Yet despite this, at least one and sometimes several of the metabolic variables measured was more than two standard deviations away from the normal mean in all but 3 of the 17 patients studied. Table XVI will make this clearer.

BAUER: How about the normals? Were they normal in all respects? They need not be, you know.

NICHOLS: That is perfectly true. Data for eight normals are shown. Since this table was prepared, we have done three additional normal subjects.

If they are included in the means they are very similar, as are the standard deviations. The latter are a bit smaller, but not much. The total normal group now ranges in age from 17 to 43 years and includes five men and three women. All were patients hospitalized for surgery which involved the skeleton, but who did not have any evidence of systemic bone disease. Nor were they people with recent fractures; this is important, as we have since discovered.

FREMONT-SMITH: Why is this important?

NICHOLS: That they did not have fractures is important because the presence of a recent fracture, even at some site remote from the biopsy area, seems to stimulate bone metabolic activity.

BAUER: It seemed, from table XV, that every osteoporotic individual studied was abnormal, i.e., outside the normal range, in every one of the variables studied. How abnormal were the nonosteoporotic subjects?

NICHOLS: This is a compilation of all the normal data available

at the time. The gaps occurred because of broken tubes, insufficient bone to measure everything in a given individual, and so forth.

HEANEY: In which way were they abnormal? Were they just outside the standard deviation in both directions?

NICHOLS: Yes, and this is what we are going to discuss in table XVI.

MACDONALD: Are these biopsies?

NICHOLS: Yes; the majority were taken from the iliac crest.

HOWELL: How many grams of bone does it take for that battery of studies?

TABLE XVI

OXYGEN CONSUMPTION

Sample no.	Lactate	Cells		Collagen	
		Glucose	Proline	Glucose	Proline
	Low				
32	n	n	+	−	±
42	n	n	+	−	n
44	n	n	n	n	n
48	n	n	n	n	n
	Normal				
21	n	−	n	−	+
29	+	−	n	−	+
30a	n	−	+	−	+
30b	n	+	+	n	n
34	n	−	n	−	n
38	n	n	n	n	n
46	n	+	+	n	n
49	n	n	n	n	n
50	n	+	+	n	n
53	n	+	+	n	n
	High				
24	n	−	n	−	+
35	+	n	+	n	n
40	n	n	n	n	n
71	n	n	n	n	n

NICHOLS: When we started these studies we needed about 1½ grams. Recently, we have refined our methods so that we can do most measurements with 300 milligrams. If collagenase activity is also measured, we need about 500 milligrams. There are some additional problems that arise when the sample size is very small. For example, the total cellularity of this tissue is quite low, so there is a risk of not having a representative sample of the several types of cells; the total number of cells is too small. At least this seems the best explanation for the increasing variation one gets as the sample size decreases.

Faced with the kind of scattered data shown in table XV, the next step is to see whether clear patterns emerge when the patients are classified on the basis of one or another metabolic variable. The next three tables illustrate such possible classifications.

Table XVI shows what happens when patients are grouped according to bone-cell oxygen consumption. Three groups can be distinguished with low, normal, or high values depending on whether their values fall within, below, or above two standard deviations on each side of the mean value found in samples from normal subjects. Although the majority fall within the normal range, four subjects with low rates of O_2 uptake and four subjects with increased rates can be identified. However when the results of other metabolic studies are examined, no very consistent pattern is found. About all that can be said is that when O_2 uptake is low, collagen biosynthesis is apt to be normal or low, while the label retained in cells is generally normal or (as in two cases) increased. In patients with normal bone-cell O_2 uptake, other data also tend to fall in the normal range. What changes are present tend to be above the normal range; the same is true when O_2 uptake is abnormally high.

Clearly, this classification does not bring out any very well defined patterns of change. Similarly, classification based on rates of collagen biosynthesis from glucose (table XVII) or proline (table XVIII) fails to identify any clear-cut pattern of disturbed metabolism. Again, most of the patients fall into the normal category of each classification.

When other classifications are tried, the results are equally disappointing. Indeed, the only common denominators which appear are that cellular-label retention from both glucose and proline is either normal or high, even in people with normal or low rates of collagen synthesis from the same precursor.

Incidentally, several of these patients had received sodium fluoride treatment for upward of a year at the time of biopsy. One patient was studied both before and after 1 year of treatment with 100 milligrams sodium fluoride by mouth each day. It was interesting that the metabolic patterns in these patients could not be distinguished from the

TABLE XVII

COLLAGEN SYNTHESIS FROM GLUCOSE

Sample no.	O_2 uptake	Lactate	Cells		Collagen from proline
			Glucose	Proline	
	Low				
32	−	n	n	+	−
42	−	n	n	+	n
	Normal				
30b	n	n	+	+	n
35	+	+	n	+	n
38	n	n	n	n	n
40	+	n	n	n	n
44	−	n	n	n	n
46	n	n	+	+	n
48	−	n	n	n	n
49	n	n	n	n	n
	High				
50	n	n	+	+	n
53	n	n	n	+	n
71	+	n	n	n	n

rest. Some changes did occur, however, in patient 30 (see table XV) during her year of treatment, a period over which she developed roentgenographic evidence of fluorosis of the skeleton.

The question, of course, is "What do these data mean?" My answer at present is, unfortunately, "I do not know," but perhaps you will be able to help me understand them.

URIST: May we call for some questions now about what you have covered so far?

NICHOLS: I was going to ask Dr. Heaney or Dr. Bauer to tell me how they would attack the problem from here. I find myself at a loss.

HEANEY: Before you call for questions, I would say your statistics are unmanageable. There is nothing in your presentation that could not be accounted for by random chance; this is not to say that real

TABLE XVIII

COLLAGEN SYNTHESIS FROM PROLINE

Sample no.	O_2 uptake	Lactate	Cells		Collagen from glucose
			Glucose	Proline	
	Low				
32	−	n	n	+	−
	Normal				
30b	n	n	+	+	n
34	n	n	−	n	−
35	+	+	n	+	n
38	n	n	n	n	n
40	+	n	n	n	n
42	−	n	n	+	−
44	−	n	n	n	n
46	n	n	+	+	n
48	−	n	n	n	n
49	n	n	n	n	n
50	n	n	+	+	n
53	n	n	n	+	n
71	+	n	n	n	n
	High				
21	n	n	−	n	−
24	+	n	−	n	−
29	n	+	−	n	−
30a	n	n	−	+	−

forces were not involved. I am merely talking about interpreting the data.

NICHOLS: I have also come to this conclusion.

RAISZ: I would like to ask about the problem of age. Your normals ranged from age 17 to 43 years, and I think the patients were all older except for one patient of 41. Was this a lady who had had her gonads removed?

NICHOLS: Surprisingly enough, it was a man.

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RAISZ: The question remains as to whether there is variability with age in these measurements.

NICHOLS: We cannot entirely determine this. We suspect that very young people have a much faster bone metabolic rate; but we have relatively few data from children, and most of these are abnormal children, because it is very difficult to get a normal child's bone in adequate quantity. The few samples that we have studied show variable higher rates, sometimes as much as threefold or fourfold the adult normal as defined by these numbers.

HOWELL: You refer to DNA as a standard of reference?

NICHOLS: Yes; DNA.

FREMONT-SMITH: What with automobile accidents and all, I should think you would have a lot of normal bone available, even from children.

NICHOLS: The problem is that people are rarely autopsied until some hours after death, and by this time the tissue has deteriorated, unfortunately.

FREMONT-SMITH: But people are operated on; amputations and all kinds of things are happening. There would seem to be a real opportunity if one could alert a group of surgeons to the kinds of specimens you want and to what these specimens should be put into.

HEANEY: I think, Dr. Fremont-Smith, it would be very important to work entirely with bone from exactly the same region. We have enough variability without taking a hit-and-miss sample from a leg here, a finger here, and a finger there.

FREMONT-SMITH: There are quite a lot of legs, and I suspect one would have not four to seven normals but perhaps 40 or 50 normals in a few years. One of the situations evident here is that the sample of normals is completely inadequate to base anything on. I am sure a careful effort with accident cases coming into operation would provide at least a better sample of normals than Dr. Nichols has.

NICHOLS: Obtaining good normal materials is indeed the most critical issue at this time. Dr. Peck, did you have a question?

PECK: I would like to ask about the effect of anesthesia on the metabolism of surviving cells.

NICHOLS: I do not know.

PECK: Do you have any patients that were operated on under various types of anesthesia?

NICHOLS: The biopsies were obtained under a variety of anesthesias, most of them general anesthesia. I have not examined the data in relation to the anesthetic agent; it should be done.

BAUER: What, by definition, do you mean by "osteoporosis"? For those who do not work in this field, may I explain that there are several current definitions of osteoporosis; whatever definition one chooses, it is

still difficult to distinguish between osteoporosis and nonosteoporosis.

NICHOLS: This group was defined as follows; they had pain.

BAUER: Where?

NICHOLS: The patients had crushed vertebrae, as shown by roentgenographs. They had normal serum calcium and phosphorus.

BAUER: My other question is "Have you done this kind of analysis on bone biopsies in experimental animals?"

NICHOLS: We have data from rats, pigs, guinea pigs, and rabbits, but none from dogs or cows.

BAUER: Even though you do not have too much normal material (this is the same practically for whatever one tries to do in studies of disease—it is difficult to get normal materials), I am sure that the biochemical methods, or other methods, used are much more difficult to handle than the logistic problem of getting normals. When you compare the changes that you find in your few normals with those of the many nonnormals, are these comparisons in approximately the same range as those in your animal experiments?

NICHOLS: The normal range appears, so far, to be wider in man than in animals, but I think this probably is a function largely of number of samples examined.

ROBINSON: In the investigations of bone that you obtain at the time of surgery, there are apt to be, particularly in the osteoporotic individual, many other cell types in the whole bone specimen besides osteoblasts, osteoclasts, and osteocytes. Do you have any way of standardizing your sample so that you know how much actual bone tissue is in this specimen of whole bone? It appears that you have a DNA ratio per gram of wet weight, and a lot of the DNA might be associated with cells that are not concerned directly with bone accretion, resorption, or maintenance.

NICHOLS: This is basically the problem of marrow contamination, which is with us in all our work with animals as well as man. The answer to your question is not entirely adequate, but perhaps if I explain the details of our procedures you will see how we try to minimize the contamination with marrow and other nonbone cells.

The biopsy, when taken, is chilled immediately in Krebs-Ringer buffer at 2° to 3° C and transported at that temperature in buffer to the laboratory; there it is carefully scraped free of soft tissue. Iliac crest samples, at least, are a mixture of a thick plate of cortical bone with trabecular bone attached. Both are finely minced with a bone rongeur and then shaken quite vigorously with cold buffer. By the way, Dr. Talmage tells me that this is the way he gets osteoclasts out.

TALMAGE: Roughly.

NICHOLS: We use this technique to wash out cells that look like marrow on smear. If washed three times, a piece of bone which

was red is now pale pink or white and on histologic examination has lost most of its marrow elements.

ROBINSON: And then you weigh that material?

NICHOLS: Right. Then after incubation, the cell fraction is separated chemically.

BAUER: Have you had an opportunity to compare your data obtained on biopsy material with data obtained on autopsy material; i.e., from individuals who have been dead for 24 hours?

NICHOLS: Actually, we have done only two post mortem samples, both traumatic. Neither showed much, if any, activity, as I recall. However, this was some time ago, and perhaps we should go back and try again.

Paget's disease is something we have yet to study. We have looked at the influence of fractures once, in a patient with osteogenesis imperfecta. We have also examined isolated cases of other diseases. I showed you osteoporosis partly to see if somebody could help me find an interpretation of the data and partly because our findings indicate a completely polyglot group of cellular disturbances; this is important because osteoporosis is a polyglot disease.

RAISZ: I do not believe that the complexity of your data rules out a simplistic theory of osteoporosis. Although this theory may not be correct, I would like to hear the reaction of this group to the possibility that there is a single mechanism for the development of osteoporosis. I think that the variations which you have observed could occur on the basis of the heterogeneity of the population of cells that we have been looking at.

MACDONALD: Do those data not show that there is a need for a method that would establish whether the osteopenia is progressing or whether it is quiescent? Perhaps this distinction in these patients would simplify some of the comparisons that may bother you. Methods of determining whether the osteoporosis is active or quiescent might open a new area for discussion.

PECK: I think it would be very valuable, if feasible to obtain; the reproducibility of the observation in the same patient from day to day, week to week, and even month to month.

NICHOLS: This is something we have not done. Patients are reluctant to be biopsied a second time; so far I have not asked a patient for a third biopsy. The amount of bone needed is still too great and the procedure too traumatic to justify such repetitive studies at present.

HEANEY: You are to be congratulated for attempting this exceedingly difficult task; I think it has to be done. Also, I think it is this sort of approach that is going to provide us with valuable information, but I am convinced from what I have seen so far that much of the discussion of the last 2 or 3 minutes is completely irrelevant. We do not know

whether the data are themselves dispersed because we have no adequate standard of reference. I do not see how we can conclude anything except that this is a valuable first step, and we hope we can go on from here.

I think osteoporosis is a diverse disease with many mechanisms, many pathogeneses, and many manifestations, but I do not think your data show it one way or the other.

NICHOLS: I agree with you absolutely. We do not have an adequate standard of reference yet. Moreover, we do not know enough about the details of the cellular mechanisms to begin to interpret what we see.

PRITCHARD: Would you like to know the ratio of osteoblast to osteoclast activity? Suppose you took the ratio of alkaline phosphatase to acid phosphatase activity as a measure of the ratio of osteoblasts to osteoclasts. Surely, that ought to be a useful index.

URIST: In the serum or the supernatant?

PRITCHARD: In the bit of bone.

NICHOLS: We have made some measurements in other systems, but not in this particular one.

PRITCHARD: You could take the ratio and call it the Pritchard Index.

TALMAGE: The osteocyte also has these enzymes, so why should you be considering only the osteoblasts and osteoclasts?

PRITCHARD: If the index is a useful guide as to whether the bony condition is progressive or not, let us not worry too much about the osteocytes.

FREMONT-SMITH: Have you had any samples of bone from disuse, for instance, from polio cases?

NICHOLS: No; we have not. There is so little paralytic polio now that we rarely have such patients, and it is very hard to come by an adequate sample.

I would like to turn the discussion now to resorption. So far, we can only say that these patients have osteopenia by roentgenographic examination. The only thing that tells us that resorption might be increased is that one cannot make a case for anything else happening on a regular basis. However, now we have a few measurements of collagenase activity in human bone and this permits us to categorize one small group of osteoporotic individuals more precisely in terms of resorptive activity.

The essence of the technique is similar to that we have described for animal bone (ref. 158). One takes human bone, grinds cells out of it, homogenizes the cells with Triton–X–100, which breaks up the lipid membranes, and incubates this mixture with ^{14}C-labeled bone collagen for 1 hour. Then the mixture is diluted with ice-cold water

and poured into an ultrafilter. The ultrafiltrate from the incubation mixture is then dried, taken up in a small volume, and counted.

It turns out that the counts in the ultrafiltrate are in proline and hydroxyproline, when the collagen has been labeled with ^{14}C-proline. These counts are present in the same proportions as they are in collagen, and their specific activities are similar to what they were in the parent molecule, so that it seems likely we are measuring collagenolytic activity. The amount of such activity seems to vary directly with the DNA content of the homogenate in normals. The mean value obtained in five such individuals is 8.75 milligrams collagen broken down per milligram DNA, with a range of ± 2 S.D. on each side of the mean of 4.4.

At present we have two measurements in patients with osteoporosis; one was 62 years old and the other was 57. The latter is from a lady who had been treated with estrogen for some time and had typical postmenopausal osteoporosis. The other one is from a man with coronary arteriosclerosis and marked osteoporosis—possibly some form of osteogenesis imperfecta, because he had a crushed vertebra when he was only 33. The point is that these data are just enough to suggest that an elevated bone collagenase activity may turn out to be still another way of classifying osteoporotic patients.

TALMAGE: Could I ask one thing concerning your data? Were the samples from the osteoporotic individuals taken from the same part of the same bone as the samples from the control patients?

NICHOLS: Yes.

TALMAGE: All of these were from iliac crests?

NICHOLS: Almost all. There is one other thing that ought to be said; the metabolic activity per wet weight unit, as generally defined, varies greatly depending on where one takes the bone sample—head of the femur, iliac crest, or rib. However, on the DNA basis the metabolic variables do not vary much.

URIST: DNA is a valid index of cellular activity.

ARNAUD: You really do not know if some of the results obtained might actually be the result of fewer cells doing more work.

URIST: Whatever the interpretation, Dr. Nichols is quantitating the cellular activity in the sample. The method should appeal to Dr. Bauer because he has changed the name of the disorder from osteoporosis to osteopenia.

BAUER: If you have two patients both suffering from osteoporosis and one patient is a 36-year-old man and the other a 70-year-old woman, you must be very cautious in concluding that these two individuals are suffering from one and the same disease. If we define osteoporosis as a condition of too little bone and fractures, then many different

mechanisms may produce this condition. Etiologically then, the 36-year-old man does not suffer from the same condition as the 70-year-old woman.

ARNAUD: I do not think that follows at all. I think they may be the same.

BAUER: Dr. Nichols, I am most intrigued by attempts to miniaturize your bone-biopsy analyses. Do you see what I am driving at?

NICHOLS: I think so. One can just go on making measurements because one knows how, but I am not sure this is a terribly valuable approach to the problem. This is the reason that I am beginning to think that maybe the thing to do is to leave shrinking elderly ladies alone until we know a bit more about normal bone-cell processes.

URIST: Perhaps I can arouse some discussion from Dr. Currey, Dr. Sjöstrand, Dr. Bélanger, and some of the others who do not see patients with osteoporosis every day, by showing a few figures to illustrate the nature of the problem.

BUDY: For the record, would someone please define "osteopenia"? A working definition would be most useful at this time.

BAUER: Too little bone.

FREMONT-SMITH: Good.

CURREY: The sturgeon has osteopenia.

HEANEY: As long as someone asked this question, I think when we say "too little" we are referring to some frame of reference. Too little for what?

BAUER: For the normal.

ROBINSON: The definition ought to be "too little bone tissue for a unit volume of whole bone."

HEANEY: For what?

BAUER: I think the simplest is "too little bone, as compared with normal." It does not disturb me too much that this cannot be measured very accurately at present.

HEANEY: It does not disturb me that it cannot be measured out, but when we say "too little" it means deficiency somehow, and the very word "deficiency" means some frame of reference. Is it too little for structural purposes?

MACDONALD: Yes.

HEANEY: I quite agree. Is it too little for homeostatic purposes?

URIST: Besides being too little in amount, the bone in the aged patient with typical osteoporosis is defective, degenerate, in part, dead tissue. I will substantiate these statements with some figures of patients I have seen in the clinic.

Figure 86 shows a 67-year-old woman with a typical form of severe senile osteoporosis in the lateral view (left) and the posterior view (right). Note the increase in the length of arms relative to the length

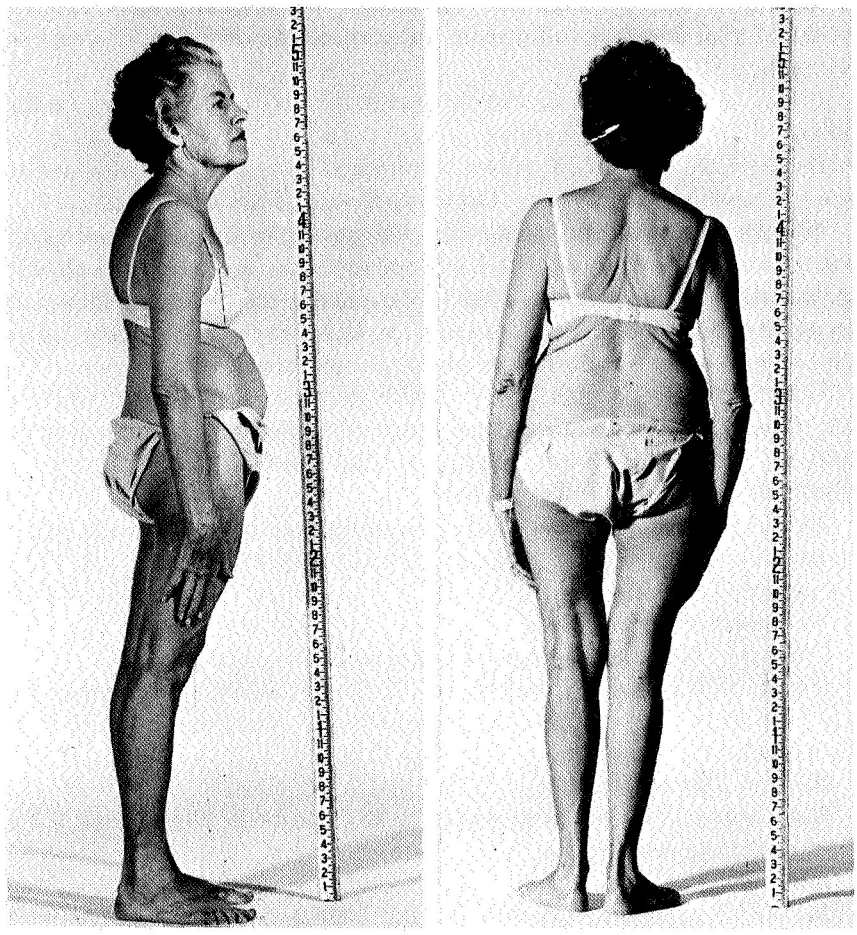

FIGURE 86. Photographs of a 67-year-old woman with a typical form of severe senile osteoporosis.

of the torso. Also, note the deep circumferential skin folds just below the costal margins. When the patient was measured with a yardstick at monthly intervals, it was clear that her height decreased progressively, and over a period of 2 years she lost approximately 3 inches in the length of the torso.

In view of the location of the gross changes in the spinal column, the best place to do a biopsy on a patient with osteoporosis would be on a vertebral body. Unfortunately, this is not readily accessible or suitable for obtaining an ample specimen. The next best place is the proximal end of the femur on the lateral aspect or the medial aspect of the upper tibia. It is customary to assume, but by no means proved, that the disease is qualitatively the same in all parts of the skeleton.

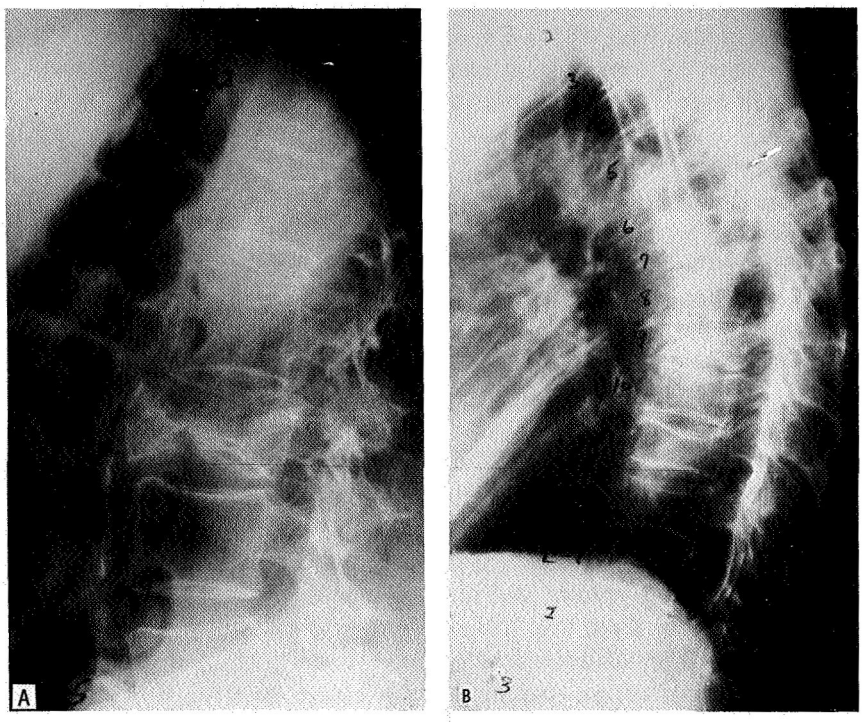

FIGURE 87. Roentgenographs of osteoporotic spines: (*a*) Lumbar spine and (*b*) dorsal spine.

Figure 87(*a*) is a roentgenograph of an osteoporotic lumbar spine showing ballooned disks, thin anterior cortex, collapse of the third lumbar vertebra, and balloon-shaped intervertebral disk spaces. Figure 87(*b*) is a roentgenograph of the dorsal spine of a typical patient with senile osteoporosis. Note the collapse of the 6th, 7th, 9th, and 11th thoracic vertebrae and the upper dorsal kyphosis.

A reduction in trabecular bone mass, as well as cortical bone, occurs and can be determined by measuring the density of the vertebral body as an organ (apparent vertebral density).

The procedure on autopsy specimens, as employed by Dr. Michael Gurvey, a postdoctoral fellow in the Bone Research Laboratory at UCLA, is as follows: (1) the vertebral bodies are scraped clean of ligaments and weighed on an analytic balance, and (2) the volume of the vertebral bodies is determined in water, by use of the volume of water displaced by the bone, expressed in cubic centimeters. The specimens are then prepared and analyzed for apparent density as follows:

1. The core of the center of the vertebral body consisting of only spongiosa is removed with a 2.6-centimeter hole saw.
2. The core of spongiosa is washed under a stream of cold water to remove all soft tissue elements (bone marrow, vessels, blood, and so forth); it is then cut into disks of 1 to 2 centimeters in thickness with a band saw.
3. The disks of spongiosa are agitated for 24 hours in cold tap water on a shaking machine to remove soft tissue cells. When the process is complete, the color changes from yellow to white.
4. The disks of washed bone are defatted in a Soxhlet apparatus by refluxing for 24 hours in a 2-to-1 solution of chloroform and methanol; then they are dried in an oven at approximately 80° C for at least 72 hours or until they reach a constant weight.
5. After weighing on an analytic balance, the volume of each disk is determined by measuring the dimensions with a sliding caliper and using the formula, r^2 times height; the volume is expressed in cubic centimeters.
6. Apparent density of the spongiosa disks is then calculated from the weight per unit volume and in grams per cubic centimeters.

Five gradations from five different subjects are shown in figure 88. Only the subjects with bone density of 0.128 and 0.107 had spontaneous fractures, or pathologic osteoporosis. These patients had approximately 40 percent of the bone mass, or apparent density, of the average nonosteoporotic young individual at age 30.

Using the method described in the preceding paragraphs, the vertebrae were used to construct a spondylometer for use in patients with osteoporosis. A spondylometer is defined as a scale for comparing normal and abnormal radiopacity with the aid of the naked eye; it reveals the distribution, quality or structure, and quantity of bone tissue. Figure 89(*a*) is a roentgenograph showing the spondylometer and the lumbar spine of a 50-year-old nonosteoporotic woman. The radiopacity of the second lumbar vertebra corresponds approximately to the bottom vertebra, or to the most dense on the column of the spondylometer. The vertebra in the top of the spondylometer is from an osteoporotic patient and shows deformity from an old fracture. Figure 89(*b*) is a roentgenograph of the spondylometer and the lumbar spine of a 70-year-old woman with severe osteoporosis. The radiopacity of the first and second lumbar vertebrae correspond to the radiopacity of the top two segments of the spondylometer that were removed from autopsy subject with severe osteoporosis. The first lumbar vertebra has collapsed, while the second is biconcave.

We have also performed biopsy studies on patients with severe osteoporosis before treatment to exclude other disorders, such as osteo-

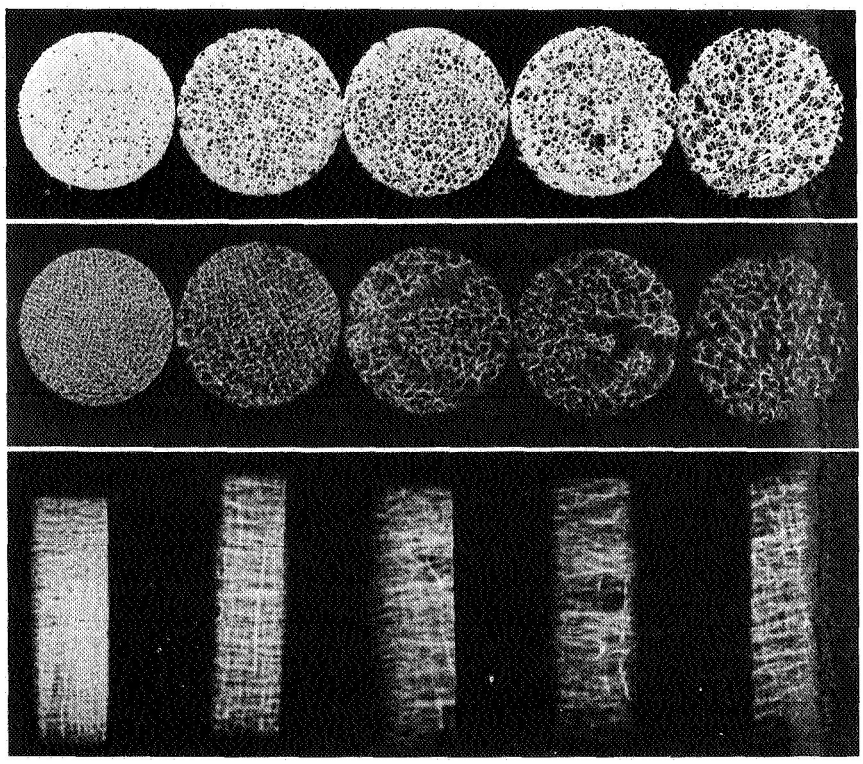

FIGURE 88. Photographs (top row) and roentgenographs (center and bottom rows) of specimens of bone from osteoporotic subjects taken from center of vertebral bodies with apparent densities (left to right) of 0.256, 0.243, 0.157, 0.128, and 0.107. Note that the trabeculae are not simply reduced in number. The vertical trabeculae increase in thickness as the horizontal trabeculae are resorbed. These changes in the structure of the spongiosa occur while the cortical bone decreases progressively in thickness.

malacia, osteitis fibrosa, multiple myeloma, and so forth (ref. 105).

FREMONT-SMITH: What bone did you biopsy?

URIST: The lateral aspect of the upper end of the femur; also the medial cortex of the proximal end of the tibia in patients with severe osteoporosis of the spinal column. Unfortunately, the spinal column is inaccessible for biopsy on aged individuals. We made cell counts, which are summarized in table XIX and observed an overall difference of 6 percent more dead bone in the tibia and femur of the osteoporotic than the nonosteoporotic. The difference may be greater in the vertebral bodies of the two groups, but we do not have data to prove this.

NICHOLS: This is dead bone as judged by empty lacunae or calcified cells?

URIST: Yes.

HEANEY: Dr. Nichols, why are you reluctant to call that dead bone?

TABLE XIX

OSTEOCYTE COUNTS IN CORTICAL BONE OF OSTEOPOROTIC AND NONOSTEOPOROTIC AUTOPSY SUBJECTS

Case no.	Age	Inner lamellae [a]				Outer lamellae [a]				Interstitial lamellae [a]			
		S	R	C	E	S	R	C	E	S	R	C	E
Osteoporotic:													
1	42	3	3	1	0	1	6	3	1	0	5	0	24
2	44	3	2	1	1	1	5	4	3	0	4	1	26
3	50	3	4	0	0	1	6	3	3	0	5	0	30
4	54	3	3	1	0	2	6	3	4	0	8	0	29
5	76	3	2	1	0	1	6	5	3	1	4	0	32
6	84	3	2	1	1	1	6	3	3	0	7	1	28
7	67	2	3	2	1	0	5	4	4	0	5	2	30
8	71	2	2	3	1	1	7	5	4	0	7	0	27
Average	63	2.7	2.6	1.2	.4	1.0	6.0	3.7	3.0	.1	5.7	.5	28.2
Nonosteoporotic:													
C1	48	7	1	0	0	4	5	1	1	8	6	0	15
C2	50	7	1	0	0	4	4	0	1	8	6	1	18
C3	71	7	1	1	0	4	2	2	2	4	3	2	27
C4	80	5	2	1	1	3	4	3	2	1	2	1	28
Average	62	6.5	1.2	.5	.2	3.7	3.7	1.5	1.5	5.2	4.2	1.0	22.0

[a] Number of stellate (S), retracting (R), calcified (C), and empty (E) lacunae per field.

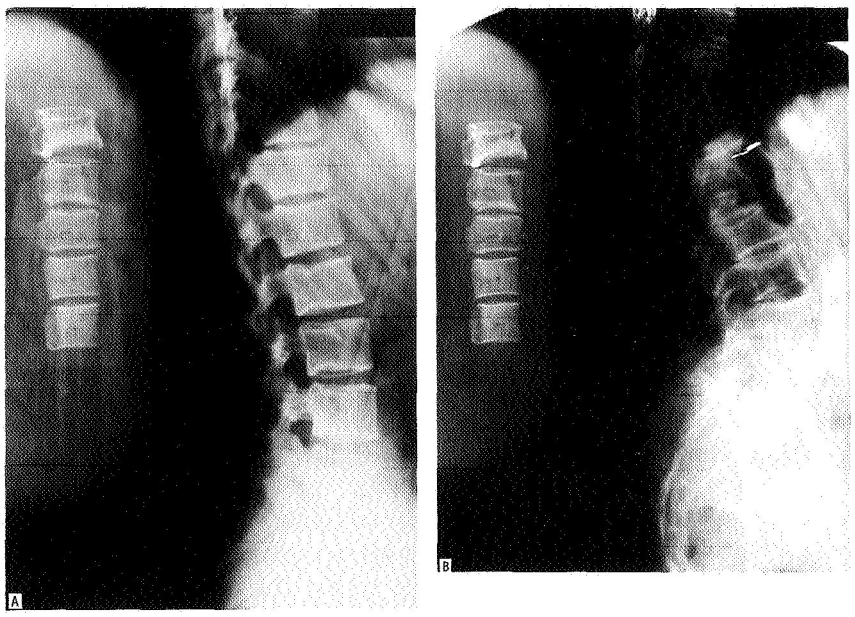

FIGURE 89. Roentgenographs of the spondylometer and lumbar spine of (*a*) 50-year-old nonosteoporotic woman and (*b*) a 70-year-old woman with severe osteoporosis.

NICHOLS: Because I think that just because a particular individual cell or set of cells is dead, it does not mean that the bone is necessarily dead any more than that it is necessarily alive when it has an intact cell in it. This is important because what we are talking about right now is a cell type that has a job to do which it is or is not doing. Cells on the surfaces may be performing quite normally while some in the depths may not, and vice versa. Really, all bone can be called "dead" because most of it is nothing but a collection of extracellular collagen which is calcified.

CURREY: Oh, no, no.

FREMONT-SMITH: The keywords were "nothing but." If you had left out the "nothing but," everybody would agree.

CURREY: By what criterion do you say there is no difference between a bone that is chock-full of healthy osteocytes and bone that is chock-full of mineralized osteocytes? Are these the same as regards life and death?

NICHOLS: Clearly, not so far as the osteocyte is concerned. I say calling a bit of bone alive or dead so far as the calcified matrix is concerned is an improper use of the term.

CURREY: But you are only talking about 99 percent of bone when you are talking about calcified matrix, and the other percent is very important.

NICHOLS: Agreed. What I am saying is that we should refer to an osteocyte as dead, rather than saying the bone is dead. Do you agree with this thought?

BÉLANGER: Oh, of course, very much so, because this sort of thing can be followed by a lot of activity at the surface, either bone growth or osteoclasia, and these two types of cells in the bone are not dead at all. The osteocytes are dead inside.

NICHOLS: I think this has great implications in how tissue behaves.

HEANEY: But that microscopic volume is certainly dead. One does not say the whole femur is dead because it has a few microscopic areas of empty lacunae.

HOWELL: Is the method of fixation and embedding such that you are not knocking a soft little particle out of a solid sheet of bone? There could be some difference in ease of removal of the soft part rather than actually not being there in the first place.

URIST: Fixation and artifacts are certainly a cause for concern. However, the empty lacunae in an osteoporotic individual are also actually enlarged. This is described in the old literature in various bone diseases as slow necrosis, oncosis, cell necrobiosis, or osteonecrobiosis. There are all gradations of oncosis. It occurs in normal aging of bone and accounts for a steady increase in the amount of dead bone in the skeleton with time. The question is whether the process is accelerated, accentuated, or exacerbated in osteoporotic compared with nonosteoporotic persons.

HOWELL: If your thesis is correct, that the axial skeleton lumbar spine is the seat of the worst disease, autopsy changes should not alter this hard-tissue phenomenon you describe, and therefore you ought to be able to document how consistent or how prevalent these changes are in the axial skeleton.

CURREY: There were two figures you mentioned, of 28 and 22 percent dead cells, which you said was not a great difference, but the two types were very consistent. This surprises me. I do not know what your standard deviations were, but for the counts that I did on healthy nonosteoporotic people, I would certainly never have been able to distinguish between 28 and 22 percent, except perhaps on an enormous series; however, you are quite happy that the variance was sufficiently small for you to distinguish between them.

URIST: The 6-percent difference is based on a sample of 10 000 cells counted by three people working independently.

CURREY: This is just in two different people?

URIST: We have biopsies on 24 cases of severe osteoporosis and on 10 cases of nonosteoporotic persons of comparable age with osteoarthritis in various joints.

PRITCHARD: This material is very difficult to fix adequately; I thought that some of the spaces you said were empty had cells in them, but there seemed to be some that showed fixation artifacts.

URIST: You are quite right; but to deal with this possibility the retracting osteocytes were counted separately, as shown in table XIX.

PRITCHARD: Were they reactive? There is the possibility of error here.

URIST: Let us go on and see more material from autopsy subjects.

BÉLANGER: May I say something on this topic of empty lacunae and dead osteocytes? This is a phenomenon which one can obtain progressively with hyperparathyroidism in animals, such as we have done with Dr. Krook at Cornell on horses. As the disease or syndrome progresses, the number of these things increases, and one can follow also the death throes, if I may say so, of the osteocytes in this type of material, so it is certainly no artifact.

URIST: McLean and Bloom (ref. 140) described osteonecrobiosis in experimental hyperparathyrodism in rats.

A good survey of pathologic osteoporosis on autopsy subjects is difficult to complete. The reason is that patients with severe pathologic osteoporosis are long-lived and healthy; therefore, they appear relatively infrequently in university teaching hospitals for autopsy. We would like to obtain data like that shown in figure 90 from 50 patients with severe osteoporosis, but we find that less than 1 percent of autopsy subjects have severe osteoporosis comparable with that found in 85-year-old white healthy females.

NICHOLS: How did you get the marrow out? Are the specimens dried first?

URIST: The marrow was removed by cold-tap-water agitation on a shaking machine, and then refluxed for 24 hours in chloroform and methanol in a Soxhlet apparatus.

PECK: Do you have any technique for measuring the interstitial volume? I should not have said "interstitial volume," I should——

URIST: We have not measured the intertrabecular interstitial volume, but that could be done.

NICHOLS: Your point is important because in our data we cannot find any difference in metabolic activity on a DNA basis between the ages of 40 and 80. If anything, the cells are a little more active in older people.

URIST: The cells may be more reactive, but in terms of the net change, bone mass diminishes with age. Reduction of bone mass with age has been measured by Trotter el al. (ref. 159) on whole bones, Arnold (ref. 160) on blocks of bone, and Jowsey (ref. 161) on microradiographs.

I think there is some—but not much—stable information in this

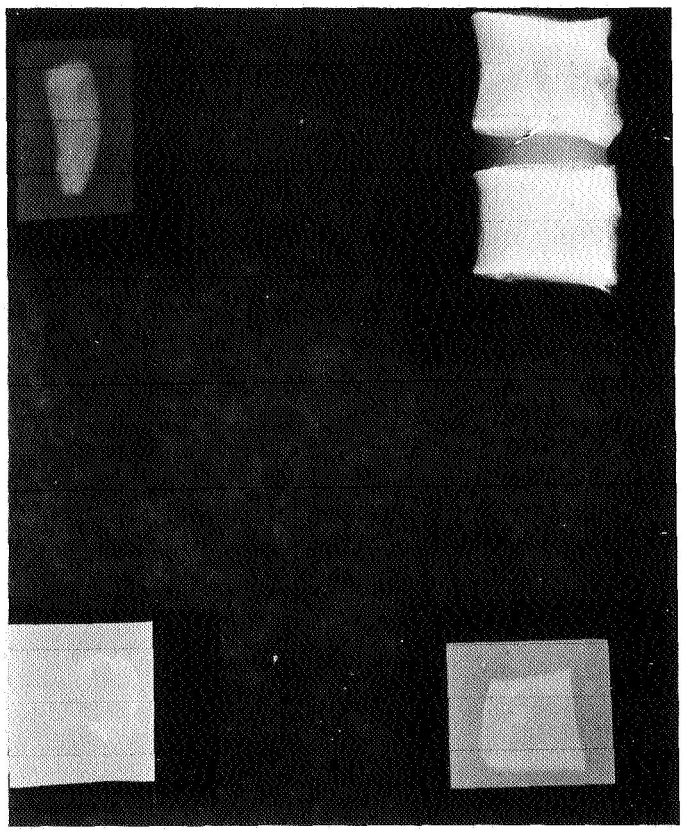

FIGURE 90. Radiographs showing a four-organ preparation from a nonosteoporotic autopsy subject, male, age 67. The tissue in the upper left is skin; lower left, fascia; upper right, first and second lumbar vertebrae; lower right, aorta. Samples of tissue from the four organs were analyzed for total calcium, total phosphorus, hexosamine, and collagen in an effort to determine whether mineral was transferred from bone to soft tissue in patients with osteoporosis. The results on approximately 39 consecutive autopsy subjects were equivocal.

field that is valuable and that we can respect. I do not think everything is chaos.

Tables XX and XXI demonstrate that approximately one of every four white healthy females, average age 85, in the United States, has severe osteoporosis; one of every seven debilitated, inactive, chronically ill white male, average age 66, has a moderate degree of osteoporosis. Statistics for healthy men in the seventh decade are not available. The period of life expectancy of the average male is less

TABLE XX

CONDITION OF THE SPINE IN 100 CONSECUTIVE WOMEN (AVERAGE AGE 85)

Radiologic observations	Percent
Generally negative, good bone density	14
Spondylosis, good or increased bone density	60
Osteoporosis (diagnosis based upon collapsed vertebra)	26
Compression fractures of dorsal spine	26
Biconcave lumbar vertebra	24
Compression fractures of lumbar vertebra	20
Compression fractures in both dorsal and lumbar spine	18
Fractures of the hip	15
Spondylosis and osteoporosis	12
Severe kyphosis with spondylosis	4
Severe kyphosis, osteoporotic (from fractures)	14
Severe kyphosis, Scheuermann's type	1

TABLE XXI

CONDITION OF THE SPINE IN 100 CONSECUTIVE MEN (AVERAGE AGE 66)

Radiologic observations	Percent
Generally negative, good bone density	9
Spondylosis, good or increased bone density	70
Osteoporosis (diagnosis based upon collapsed vertebra)	18
Compression fractures of the dorsal spine	27
Biconcave lumbar vertebra	14
Compression fractures of the lumbar spine	15
Compression fractures of both dorsal and lumbar spine	9
Spondylosis (minimal) and osteoporosis	10
Severe kyphosis with spondylosis	3
Severe kyphosis, osteoporotic (from fractures)	11
Severe kyphosis, Scheuermann's type	0

than that of the average female. The human female is one of the longest lived of all mammals.

FREMONT-SMITH: The men were much younger, were they not?

URIST: The average age was 66 years; the average for the women was 85 years.

NICHOLS: I think your group of males was unusually short-lived.

URIST: The question that arises now is whether physiologic reduction of bone density with aging or inactivity (without collapsed vertebrae), pathologic osteoporosis, and multiple spontaneous fractures are different degrees of one and the same process. Our working hypothesis

is that excessive or extensive osteonecrosis of the cortical bone of the vertebral bodies is a characteristic of pathologic osteoporosis, but we do not have sufficient data to prove it; also, we have no idea of the cause of the necrosis. Don Fareed, a medical student research fellow, is making cell counts on cortical bone and perfusing the blood vessels of autopsy specimens.

NICHOLS: Dr. Bauer has a few figures to show which complement what Dr. Urist has been talking about. Because it is clear that we do not know much about changes in biosynthesis in the etiology of this disease, we ought to take a look at our knowledge of resorption and its disturbances to see if we can learn something from the animal models now available.

BAUER: I would like to present some data on the incidence of fractures in the population of Malmö, Sweden. Figure 91 (ref. 162) shows the annual incidence per 10 000 inhabitants for fracture of the distal end of the radius in males and in females. At about age 40 there occurs a sudden dramatic rise in the incidence of this fracture in females.

Figure 92 shows that there is a distinct difference between the two sexes also as to what has caused the fracture. The figure shows the ratio of slight over severe trauma, slight trauma being defined as a fracture occurring from a fall on the floor or something similar; if more trauma was involved, then it was registered as severe. We see again a very distinct difference between the two sexes.

In figure 93 we see, in the same population, the change with age in location of fracture of the radius. With increasing age the incidence of distal fractures rose in relation to shaft fractures, and dramatically more so in females as compared with males. This again suggests that something is happening in the skeleton with age, and more so in females than in males.

Figure 94 (ref. 163) shows the incidence, in the same population, of fracture of the distal end of the radius and of the neck of the femur. There is a parallel rise in the incidence of these two types of fractures with a lag period of some 16 years.

I show the diagram (fig. 95, ref. 164) to illustrate that one cannot relate fracture immediately to fragility of bone. Fracture in general can be caused by external violence or by disease; cancer metastasis or radiation injury, for example, tend to make the neck of the femur weaker than normal. But when one is looking at data of this type, one cannot escape the conclusion that in addition to external violence and such diseases, which we can define at the present time, something is happening in the skeleton that is closely related to age, and also that this progressive age factor, fragility, is an important factor in causing certain fractures in the aged.

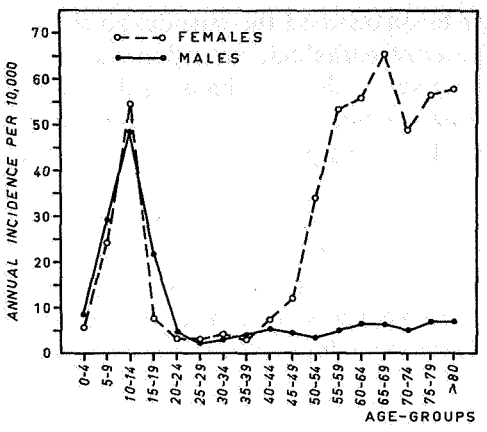

FIGURE 91. The annual incidence of fractures of the forearm according to age in Malmö, Sweden. [From ref. 162; reprinted by permission of the publisher.]

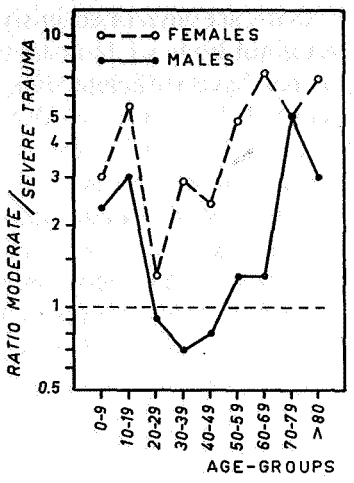

FIGURE 92. Ratio of moderate to severe trauma in males and females. The data did not permit evaluation of the degree of trauma in all cases. [From ref. 162; reprinted by permission of the publisher.]

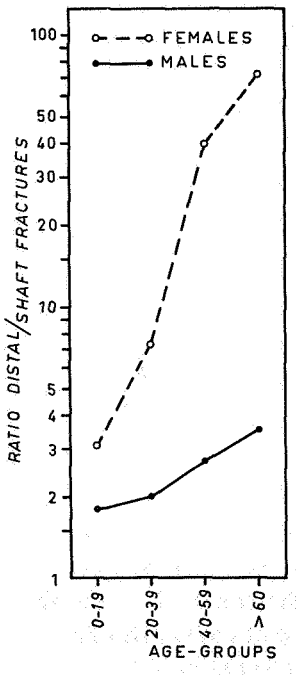

FIGURE 93. Change with age and sex in the location of fracture of the radius. [From ref. 162; reprinted by permission of the publisher.]

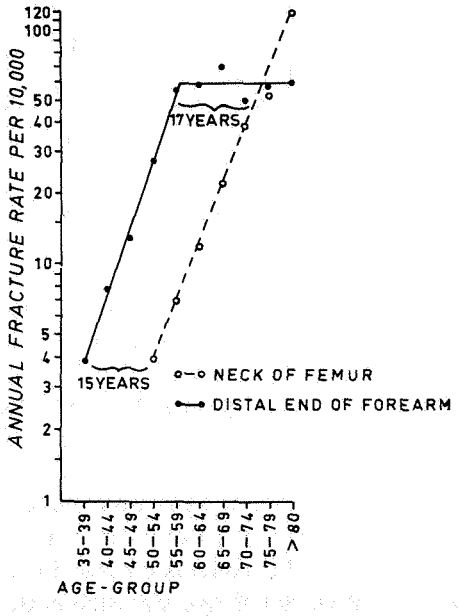

FIGURE 94. Semilogarithmic plot of age-specific rates of incidence in women of fractures of lower end of forearm and of upper end of femur. [From ref. 163; reprinted by permission of the publisher.]

If we are interested in trying to understand this age-linked fragility of the skeleton, we have to make a distinction between two mechanisms for making the skeleton fragile. One is too little bone, and the other may be, and this is relatively hypothetical, a change in the quality of the bone tissue. Unless we try to make this distinction, we will tend to overlook the possibility that fracture may occur because the skeleton is weaker than normal without there necessarily being any less bone than normal.

I submit that the epidemiologic data on fractures of the distal end of the radius in females are especially difficult to interpret solely in terms of too little bone. It is quite clear that the skeleton loses bone with age, but nobody has shown that the female at age 40 or 45 has lost enough bone to cause this rise in fracture incidence.

Therefore, I believe it is important to measure the strength of bone independent of how much bone there is.

One could then say that osteoporosis may be defined as a disease in which there is fracture from inadequate violence; one etiologic factor may be "osteopenia," meaning too little bone, and another "fragility," meaning a condition where the quality of the bone tissue has deteriorated. The question was raised: "Too little as compared with what?" Naturally, too little as compared with normal. I do not think one must show that it is bad to have too little bone compared with normal any more than one must show that it is bad to be underweight or overweight. It is true that because we can link sideropenia to definite symptoms of disease, it is beyond doubt a distinct disadvantage to have too little iron; and it is very definitely bad to have too little money, even though what is normal is hard to define. Even though "underweight" is not defined in terms of one weighing so little as to be blown into the ocean by a gale of, say, 20 miles per hour, it is possible to say that outside of two sigmas of normal, one is underweight; then one is able to determine if it is bad to be underweight. In the same way, I think one can very well talk about a condition of too little bone, even though perhaps it is difficult to measure, and even though one does not know whether it is bad to have it. I think it probably is.

NICHOLS: Then you would put osteoporosis into my category of disturbance of cell machinery; the difference being that you believe it is an acquired, rather than a congenital, disturbance; is that correct? In other words, do you think something goes wrong with the machinery so that the way in which the bone is made is wrong?

BAUER: I do not have any ideas about the etiology here.

NICHOLS: I am serious. This is a possibility.

BAUER: That is why I am so serious. I have not talked about the etiology. To give an example of what I am talking about, one could

say that perhaps the quality change comes about because of a change in polymerization or crosslinking; perhaps the collagen fibrils suddenly become tired and do not bite each others' tails as well as they should. But it is a very interesting fact that this possibility has not been discussed and even less studied.

MCLEAN: I think it should be pointed out that there is not too little bone for the metabolic functions of the skeleton, which hold up very well.

BAUER: Oh, yes.

MCLEAN: It is too little bone for the structural functions of the skeleton.

BAUER: I am trying to say that I do not know whether it is too little for metabolic function or too little for a structural function. I define "too little bone" as being less bone than normal in the skeleton, and I would like to know what structural importance this has. I am betting on the possibility that if I have less bone than normal, my skeleton becomes somewhat less strong than normal; but I keep in the back of my mind the possibility that without a quantitative change in my skeleton, qualitatively perhaps something happens which makes it more brittle.

MCLEAN: But you do agree that in osteoporosis the skeleton maintains its metabolic activity at approximately its normal level.

BAUER: Yes.

MCLEAN: It seems to me that it is important to differentiate between the inability of the skeleton to withstand strain and its ability simultaneously to keep up metabolic activity.

NICHOLS: What you are saying is that while one end of its function is OK, the other end may be in trouble.

HEANEY: But that is an assumption, and this is what I think Dr. Bauer is trying to say and has been trying to say for many years. I recall puzzling over this at prior conferences. There is too little bone in comparison with normal reference standards, and there is, associated with this, an increased liability to fracture. But it is another thing entirely to say that the too little bone causes the fracture.

BAUER: No, no. The thing is that you have too little bone. If you take, say, Dr. Urist's elderly ladies and you find that they have too little bone and they have fractures, or 26 percent of them have fractures of the spine, one should not jump to the conclusion that the reason they have fractures is that they have too little bone. It may be that there is a poor quality of bone, also.

MACDONALD: Is this not easily demonstrable by tensile-strength measurements?

CURREY: Well, there are practical difficulties. I think one could

calculate, quite reasonably, simply the reduction in the strength that you would expect from loss of bony material. I think there is no doubt that, in fact, there is also a change in the quality of the bone. The highly calcified regions are certainly centers which will shatter under impact in a way that ordinary bone would not. To separate the two, the change in the quality of the bone and the change in the amount of bone, and to separate their effects on fracturability of the whole bone is feasible, but technically very difficult. Certainly, it would be a very good thing if someone could get down to it.

BAUER: There is definite lack of correlation between attempts to test bone strength *in vitro* and the clinical evidence of bone fragility deduced from epidemiologic data. This is most embarrassing when we realize that we are talking of, by far, the most important metabolic bone disease we have. Twenty-five percent of Dr. Urist's elderly ladies have fracture of the vertebrae, and it has been shown in other series; in Denmark, for instance, about 25 percent of all inmates of homes for the aged have fractured vertebrae without knowing that they have it and without knowing that they have ever had any trauma. Fractured vertebrae is thus usually a very mild disease. But this is not so in fracture of the neck of the femur, which occurs in 20 percent of all women who reach 80. Fracture of the neck of the femur is not a catastrophe to those who have it; but it is catastrophic to society, not because these females would work very hard if they did not have fractures but because young people have to take care of them.

I submit that this is, in a way, what we are all studying; prevention of fracture in the aged is the man-on-the-moon project of bone metabolism.

NICHOLS: I agree with you, Dr. Bauer. There are some other ways of studying this problem that we ought to cover in this session.

NICHOLS: I presented the data that I did earlier because I wanted to indicate that one really cannot, from available metabolic data, make any sense out of the problem at all. I am not sure that one can do much better studying resorptive activity either. The fact is that we need some animal models, of which there are now a few. Whether these are models of osteoporosis is a matter which we might debate.

Dr. Raisz has been looking into Jenifer Jowsey's "osteoporotic" cats, and I hope he will tell us about them.

RAISZ: I would like to comment on the point about the difference between the supportive and the homeostatic functions of the skeleton. As Dr. McLean pointed out, the homeostatic function may be primary so that metabolic activity is maintained at the expense of support in some situations. I believe that the osteoporotic cat which Dr. Jowsey has been studying is an animal model for this, although I must admit

that at first I was reluctant to accept this model. While we agree that human osteoporosis may have many etiologies, I do not think we need to throw out the possibility of a common denominator. We do have an animal model, observed in many different species, for the effects of calcium deficiency. Calcium deficiency in different animals and in different growth states can produce typical secondary hyperparathyroidism or it can produce a state which is histologically typical of osteoporosis. Dr. Jowsey has been producing osteopenia in the adult cat by giving a high-phosphate, low-calcium diet; we have wondered whether this model might teach us anything about osteoporosis in man. There are two aspects of these animals which obviously require study. One is their parathyroid activity. We have been studying this activity in a number of such animals by measuring their parathyroid size and parathyroid amino acid uptake after long periods of calcium deficiency. These animals had normal fasting serum calcium concentrations. Homeostasis appeared to be preserved, while parathyroid activity was increased twofold to threefold as measured by the parathyroid activity index to which we referred earlier. This is far from the ideal measurement, and these questions will be clarified when good blood bioassays for parathyroid and other calcium-regulating hormones are readily available. Nevertheless, we wondered why parathyroid activity was increased with no change in fasting serum calcium. (We had seen small changes in fasting serum calcium concentration with diet in the rat.) This appeared to be a deranged feedback; however, Dr. Jowsey has found that these animals do have a period of hypocalcemia during the absorption of their diet. The animals are maintained fairly hungry and fed a restricted diet of meat all at one time. With this diet they get a large phosphate load and their serum calcium concentration actually falls postprandially. I have not seen any data to determine whether individuals with osteoporosis or other serious bone disease have any derangement in the stability of their serum calcium concentration; possibly Dr. Copp can explain this. Do some patients with osteoporosis or other chronic bone disease have instability of the serum calcium concentration, even though a normal homeostasis is maintained most of the time?

COPP: We found the diurnal fluctuations in plasma calcium concentration in osteoporotic individuals to be small and within normal limits.

NICHOLS: We too have done some calcium and EDTA infusion tests on osteoporotic patients and can find no differences from normal.

RAISZ: Another thing which Dr. Bélanger will comment on, is the dead-cell problem. Apparently the dead-cell incidence is high in osteoporosis and high in the various forms of that thing which ranges between nutritional secondary hyperparathyroidism, which is what Dr.

Bélanger was studying, and that thing which Dr. Jowsey calls calcium deficiency osteoporosis. I think we ought to look at these parameters more in these animals and in man.

COPP: I would like to make a comment here, because in young phosphate-deficient rats you get the same picture (ref. 165). Mineral is lost rapidly from the skeleton to provide phosphate essential for the soft tissues. They have very severe osteopenia and die because of collapse of the demineralized rib cage.

NICHOLS: There is another animal model—heparin osteoporosis—which is useful because it avoids the whole issue of hyperparathyroidism, which is a special disease but may well be the cause of the osteoporosis in the models cited so far.

BAUER: I can add that if you feed tigers on tiger hearts, they will develop severe osteopenia; or cats on beef hearts, which is a more common type of experiment.

NICHOLS: Some time ago a man was referred to us because the severity of his osteoporosis prevented his working; this man also had a coronary artery disease for which he had been receiving large doses of heparin for a long time, and it was quite well controlled. A variety of things suggested that his osteoporosis and his heparin therapy might be related (ref. 166), and this suggestion led to the animal model I would like to talk about. Figure 96 is a roentgenograph of the spines of two rats, one of which was kept anticoagulated with heparin around the clock for a month by John Asher in my laboratory. If you stretch your imagination, the one on the right looks a little less dense. As some of you know, Tourtellotte in Philadelphia, using sulfated low-molecular-weight dextran, produced more striking changes. I think his drug is probably a better agent than heparin because it induces less bleeding, but this is a peripheral problem. The point of importance is that the probable mechanism involved in the heparin-induced effect has now been worked out (ref. 167).

If you remember my diagram outlining the biochemistry of the bone cell, lysosomes containing collagenase were mentioned. These, it turns out, are lysed by heparin *in vitro* as is shown in figure 97. Heparin at 50 μg/mg has little effect, but at 2500 there is clearly release of collagenase from the large-granule fraction of bone-cell homogenates which contain these lysosomes. Whether there is truly an inhibition at 10 000 μg/ml, I am not sure.

Since, from these observations, it appeared that heparin releases the latent collagenase in bone-cell lysosomes *in vitro*, we wondered whether the changes in bone density in chronically heparinized patients or animals might be related to some effect of the heparin on the stability of lysosomes in their normal location in cells *in vivo*.

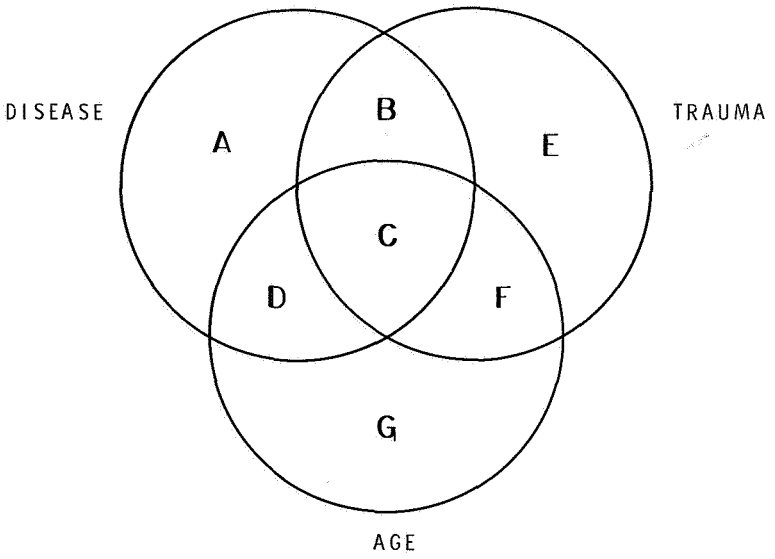

FIGURE 95. Diagrammatic representation of etiologic factors in fractures of the proximal end of the femur. [Adapted from ref. 164; reprinted by permission of the publisher.]

The results of our investigation of this possibility are illustrated in figure 98. Two sets of data are shown for lysosomes derived from bone of normal and heparinized animals. Saponin-induced activity is equivalent to the total activity present in the fraction, while "spontaneous" activity is that released spontaneously during incubation, presumably because of lysosome leakage or rupture. The ratio between the two can be considered an index of the relative resistance of the lysosomes to mechanical trauma. Simple inspection shows that heparin treatment increases total collagenase activity and the relative as well as the total amount released spontaneously. In other words, the bone-cell lysosomes in heparin treatment are more easily lysed (ref. 168). Since increased collagenase goes with increased resorption (ref. 169), these data give us an explanation for this type of osteoporosis in which no change in biosynthetic rate occurs. In contrast, one cannot demonstrate a change in the stability of the lysosome at all in parathyroid-treated animals, although one can demonstrate more total collagenase activity in the lysosome fraction.

So this is another animal model which is now available for study. This brings us to considering how bone is resorbed. Dr. Bélanger has some figures of what happens in osteolysis, a phenomenon which may hold some keys to understanding osteoporosis.

BÉLANGER: I wonder if you would like to see some figures which demonstrate the phenomenon that I mentioned a moment ago and how this fits into the animal model.

FREMONT-SMITH: Yes, by all means.

BÉLANGER: "Osteolysis," an old word which we find in dictionaries, but with various definitions, we would now like to reserve for an activity which takes place in the depth of the bone and an activity which is, it appears, related to the osteocyte at the time when this osteocyte acquires its maturity.

This sort of activity can be seen in trabecular bone as related to the center of the trabecula, and it is manifested by an enlargement of the lacuna.

When we do autoradiographs of thymidine-labeled bone we know that the thymidine appears first in the precursor cells which are outside the bone trabeculae. Then it passes out into the osteoblasts, then to the osteocytes of small size which are near the surface, then into the large osteocytes which are in the center of the trabecula, and finally this radioactive label of DNA disappears completely.

The time required for these labeled cells to go from the osteoblast stage to the last stage of mature osteocytes, of course, varies from one area to another of the body and varies with the age of the animal. The younger the animal, the faster this takes place. In the vertebrae of a rat, this lifespan of a bone cell is approximately 4 days.

YOUNG: How do they move through bone in 4 days?

BÉLANGER: Figure 99(*a*) is what we call an alpharadiograph of soft tissue. In this case, we have removed the mineral and have obtained, through bombardment with alpha particles, a density image of the tissue. The periosteum and the bone trabeculae are shown, and we can see now that the density of the organic matrix is far greater at the periphery than at the center of the trabecula, which is contrary to the degree of mineralization. We recognize this large lacuna in the center of the trabecula.

Now, as Dr. Young asks, "How do things 'move'?" We think that they move because large osteocytes, before they die, are actually perfectly capable of producing proteolytic enzymes which destroy the matrix around them and release the mineral, so that there is a continuous new stream of bone material from the surface to the center of the trabeculae as these cells die out, as the matrix is broken down and as the mineral is released. And so the bone which is here at any one time is not the bone which was there half an hour earlier.

Just to show the affinity of these effects, here is some material that I have obtained from Dr. Copp. Figure 99(*b*) represents a parietal bone of a sheep in which we can see these spaces, and then lacunae

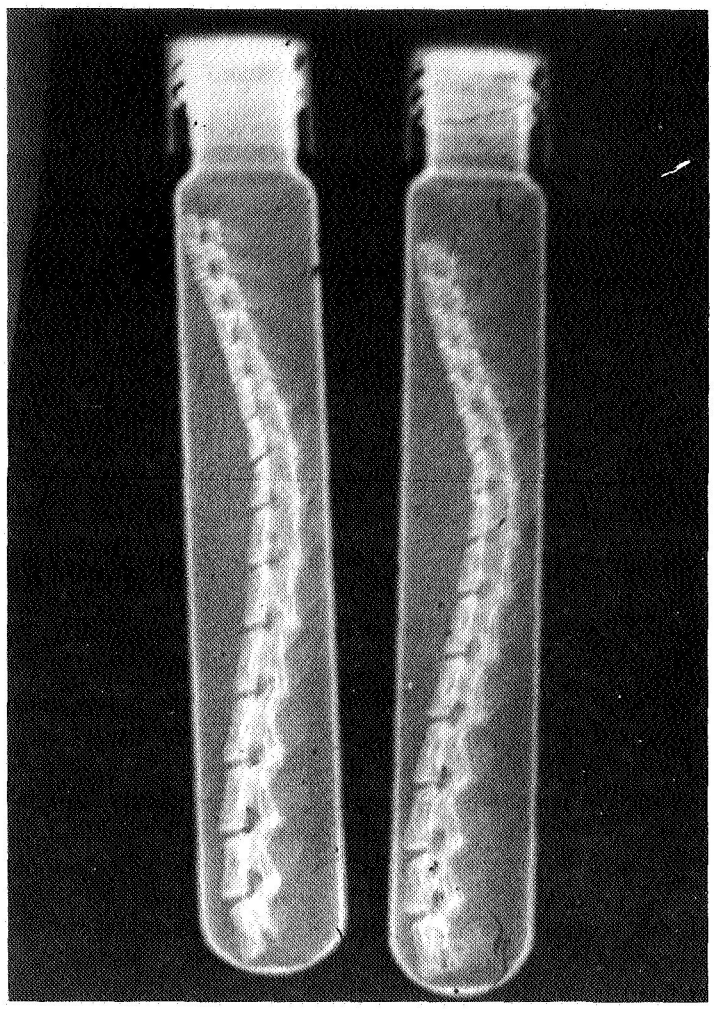

FIGURE 96. Roentgenograph of spines from two rats: Control (left) and anticoagulated with heparin (right).

with osteocytes which are either small or large in a normal sheep.

Figure 99(*c*) shows bone from a sheep that had been infused with EDTA intravenously for a period of 4 hours. We see again a very large number of large lacunae.

In figure 100, corresponding to the presence of these large lacunae, we also can see, after toluidine blue staining, that there is an increased amount of metachromatic material, both inside the lacunae and outside in the matrix which surrounds these cells. This was the first phenomenon which we observed, and we attached considerable importance to the presence of these acid mucopolysaccharides, thinking that they had

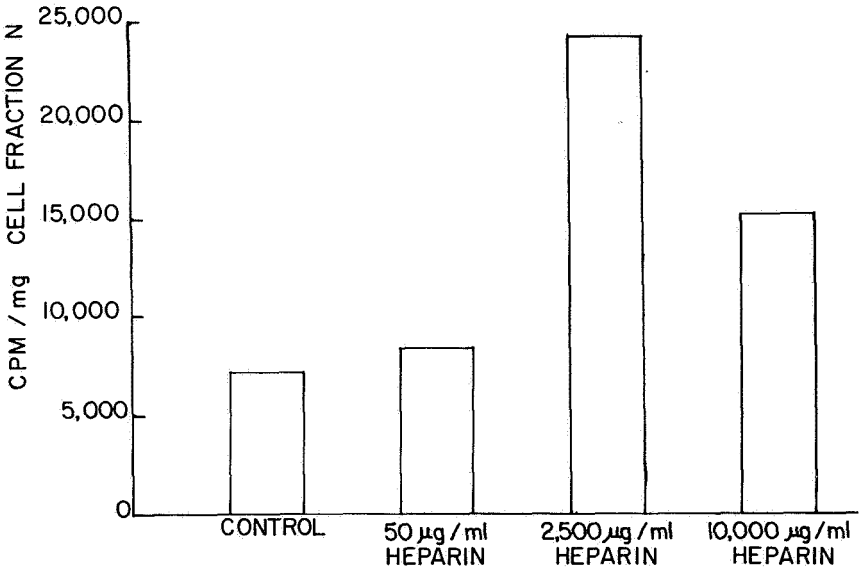

FIGURE 97. *In vitro* effect of heparin on large-granule collagenolytic activity.

a great deal to do with changing the pH level and releasing the mineral.

CURREY: Have you got a control picture to that one?

BÉLANGER: No; I do not have.

CURREY: This is rather the same point Dr. Pritchard brought up.

BÉLANGER: This has been in the literature so long I did not think anybody would be interested; this has been in the literature for many years.

BAUER: It still may be true, though.

BÉLANGER: Oh, yes; right.

PRITCHARD: Dr. Bélanger, there is another possible explanation. The first bone laid down under the periosteum, or the first bone laid down in the metaphysis, is of different character and quality from the bone laid down a few days later. The first bone laid down has irregular, coarse fibers and big cells. The later bone that buries the first bone has finer, more regular fibers and small cells. Now, how can one be sure that this is a dynamic process and not just two kinds of osteocytes?

BÉLANGER: This is an adult animal, and in the work which we did with labeled cells we know that this bone has been totally replaced in 3 or 4 days.

YOUNG: Figure 101 shows a region of woven bone and a portion of lamellar bone. The question that some of us raise is whether those cells in the center of the trabeculae you showed, those cells that have

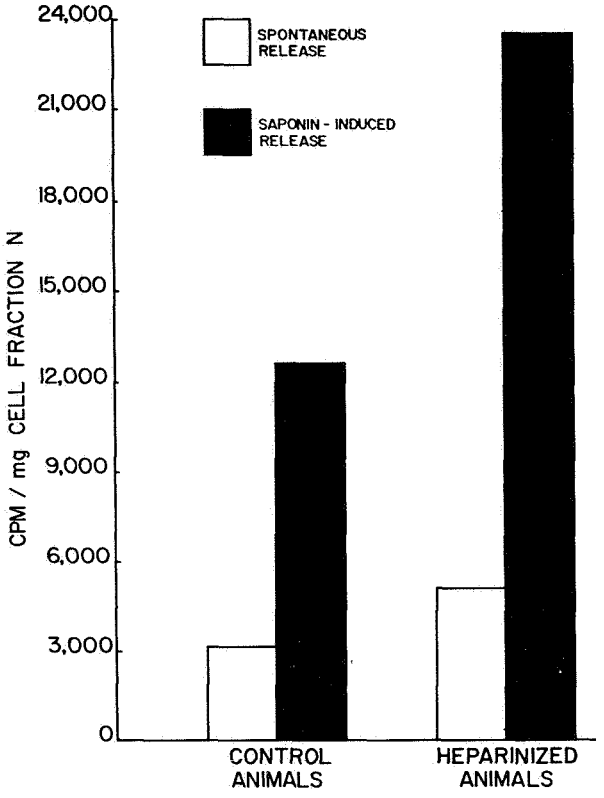

FIGURE 98. Large-granule collagenolytic activity.

enlarged lacunae and are stained differently, simply may not represent the larger osteocytes characteristic of woven bone, rather than osteolysis.

BÉLANGER: I do not worry too much about that particular point, because we later came up with a more important observation. This factor may be more significant because it may contribute to the destruction of the organic part of the matrix. We took photographic plates that have been blackened inside and processed. Over these we put a drop of trypsin and placed pieces of different types of tissue in a buffer of pH 7 on the film. We put a piece of pancreas, sections of fresh bone, and a section of bone that had been fixed in formalin for 5 minutes on the film. The pancreatic tissue made a hole in the film. The formalin-fixed bone did nothing. The fresh-bone sections made a considerable hole in the gelatin, tending to show, I believe, that this little piece of bone actually contains an enzyme capable of doing the same thing as the pancreatic tissue or trypsin.

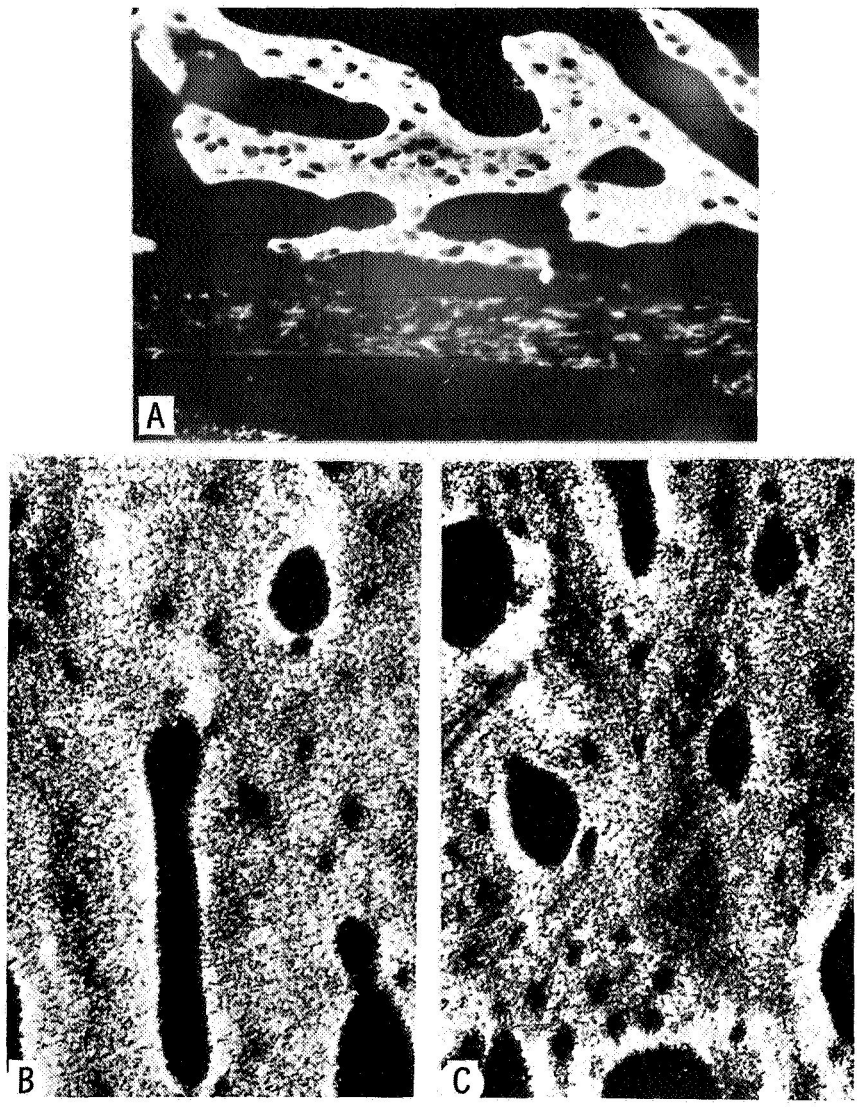

FIGURE 99. Alpharadiographs of demineralized bone sections. (*a*) Dog vertebra: Note the dense matrix of trabeculae as compared with periosteum (bottom); note also the enlarged lacunae and osteolysis in the trabecula (center). 114×. (*b*) Parietal bone of a normal sheep: Areas of low density in the matrix are seen in the vicinity of the larger lacunae. 182×. (*c*) Parietal bone of a sheep infused with EDTA for 4 hours: The large lacunae are more numerous and confluent in some areas. 182×.

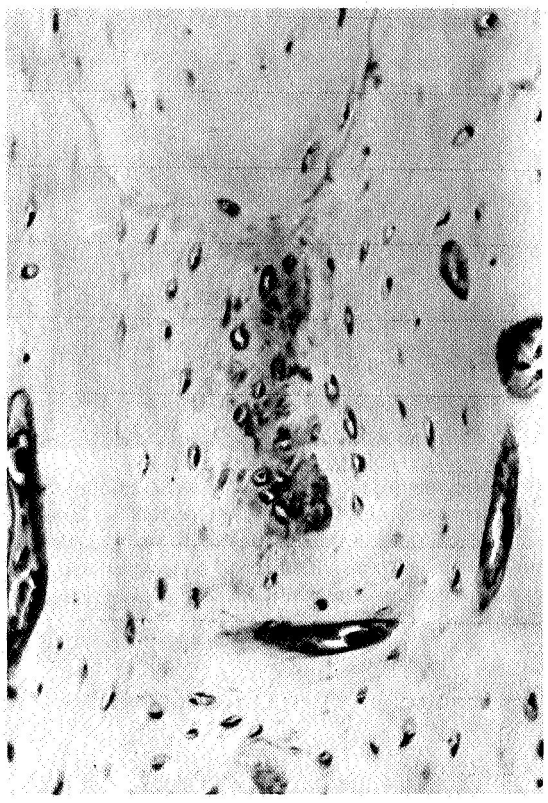

FIGURE 100. A portion of the tibial diaphysis of a horse on a high-phosphate diet for 7 weeks. Note the large osteocytes surrounded by metachromatic matrix. 136×.

When we do this sort of thing on actual section it is possible to pin-point the site in the bone where this proteolytic enzyme, or enzymes, come from.

URIST: Is this a histochemical preparation that is analogous to what Jerome Gross and George Nichols observed with quantitative methods on tissue cultures and explants?

BÉLANGER: Yes; that is correct. This is the so-called reaction of Adams and Tuqan (ref. 170), which was devised in England and makes use of the same principle of breaking down gelatin.

PECK: The ability to break down gelatin is the property of many, many enzymes. The ability to break down native collagen is a prop-

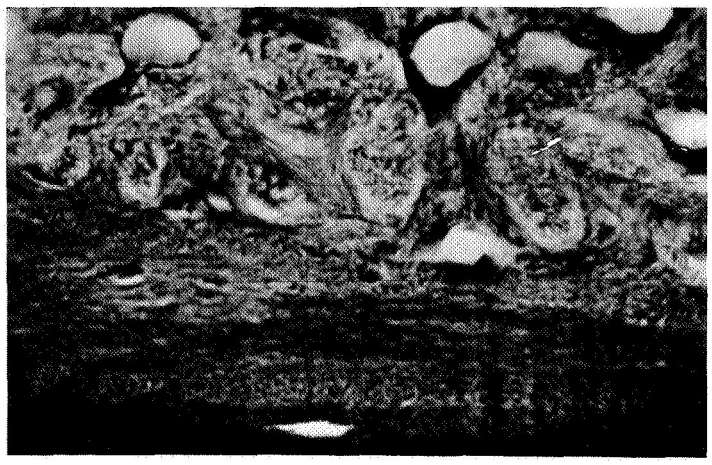

FIGURE 101. Region of parietal bone from a 26-day-old rat. Note that in the woven bone (top) the osteocyte lacunae are large and closely spaced, and the intervening collagen bundles are irregularly arranged. In the lamellar bone (bottom), the lacunae are smaller and farther apart, and the collagen bundles are more densely packed in regular array. Gomori silver stain. 1250×.

erty of collagenase; this is what Dr. Gross and Dr. Nichols have demonstrated.

BÉLANGER: Of course.

URIST: I understand that this is not collagenolysis, which is something else. I also understand that when you use the term "collagenase" you are talking of an enzyme that breaks protein down to amino acids, but when you are talking about collagenolytic enzymes it is not necessarily so.

NICHOLS: When you talk of collagenase at present, you are talking about an enzyme which works to break down a native collagen molecule (the "nativeness" of the collagen molecule needs definition in its own right) into reasonably large peptides. The further degradation of those peptides probably depends, as far as we understand the process at the moment, on a peptidase; but the question of whether this is the same enzyme as these more nonspecific proteases, which are clearly present in the cell and are active both at neutral pH (ref. 169), remains unanswered.

BÉLANGER: When you do this sort of thing on the bone section, this is actually a unicellular response which we get in the center of trabeculae where each of these large osteocytes is located (fig. 102). There is a ring of tissue destruction immediately surrounding the lacunae there.

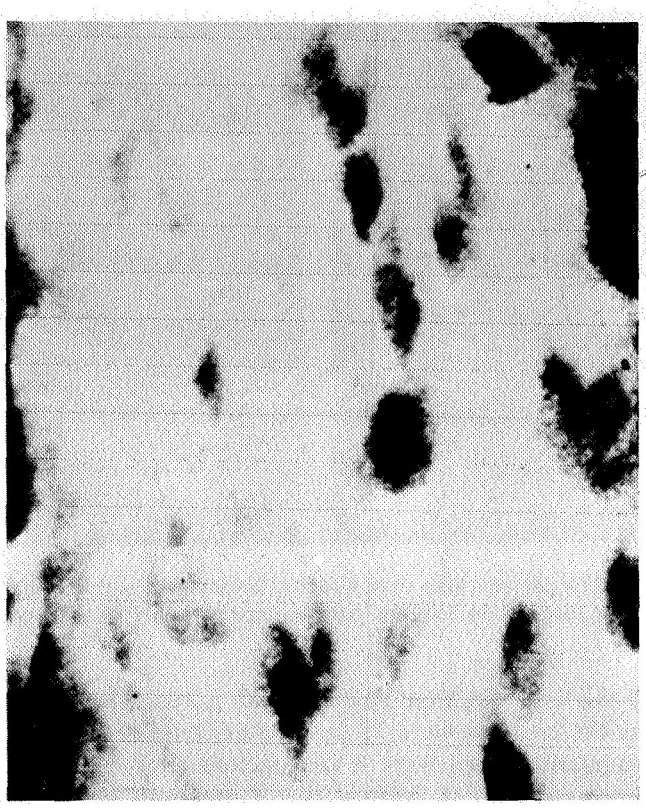

FIGURE 102. Microradiograph of an undemineralized section of the femur of a frog. Note the low density of perilacunar bone. 520×.

Figure 103 is a gross type of operation that we made last summer, using frog bones. These are femurs and humeri from two tadpoles that had been injected with a single dose of 5 units of PTE. You can see by the size of the reaction which we have recorded on the film that, indeed, PTE has increased considerably, on the average, the production of protease by these pieces of bone. If we drew these out, cut the drawings and weighed them, the proportional increase could be obtained; this has been found to be sixfold.

That this might also be related to the production of collagenase—and this is where we join our friends Gross and Lapière in Boston—is suggested by the fact that these tadpoles have been injected with parathyroid hormone. We have measured the total length of the animal after 1 day of parathyroid-hormone treatment and compared it with controls. Starting with a length of 12 centimeters for the total tad-

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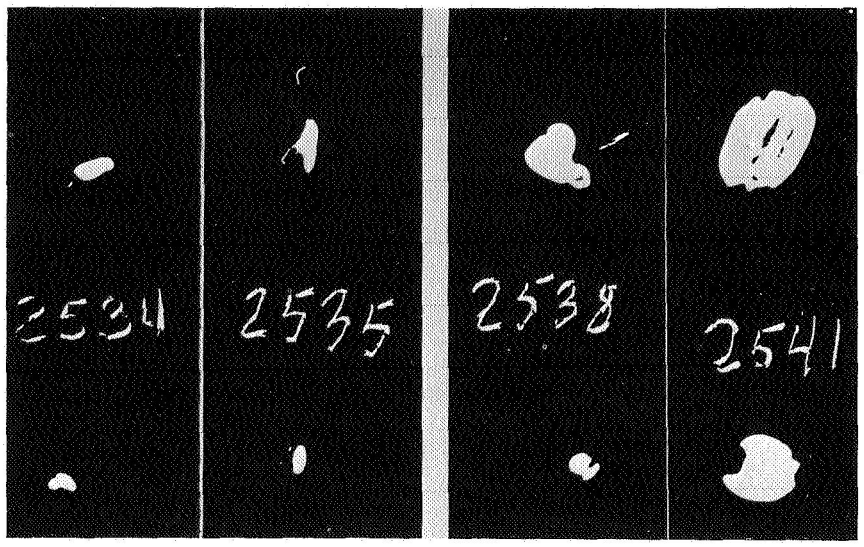

FIGURE 103. Protease reactions from bisected femurs (top) and humeri (bottom) of normal tadpoles (two on the left) and tadpoles treated with PTE (two on the right).

pole—and this is at the time when the tail is resorbing very rapidly—after 1 day the controls were 11 centimeters long and the parathyroid-hormone-treated animals were 9 centimeters long. So it is from the tails of these tadpoles, I believe, that Gross and Lapière (ref. 171) isolated collagenase. I think that the collagenase is affected by the system, as well as the protease, which we have demonstrated in the bone cells.

If you would be interested to see the cells that produce this enzyme, we have some electron micrographs.

NICHOLS: I think it should be pointed out in confirmation of Dr. Bélanger's ideas that Gilbert Vaes, working in Gaillard's laboratory, has measured the release of various bone lysosomal enzymes in tissue culture and found 8 to 10 times more enzyme when parathyroid hormone was added to the culture medium. Moreover, the total enzyme content of the culture medium after incubation was far beyond the enzyme content of the tissue originally transplanted, so that this phenomenon is clearly representing a stimulus of biosynthesis and not just increased release of preformed material (ref. 172).

BÉLANGER: These are micrographs from 2-day chick embryonic tibias, a system which actually replaces itself very rapidly. First I will show some figures belonging to Professor Baud (ref. 173) from Geneva and published in 1962, where he showed that in bone he could see osteocytes in what he called smooth lacunae, and these were at the

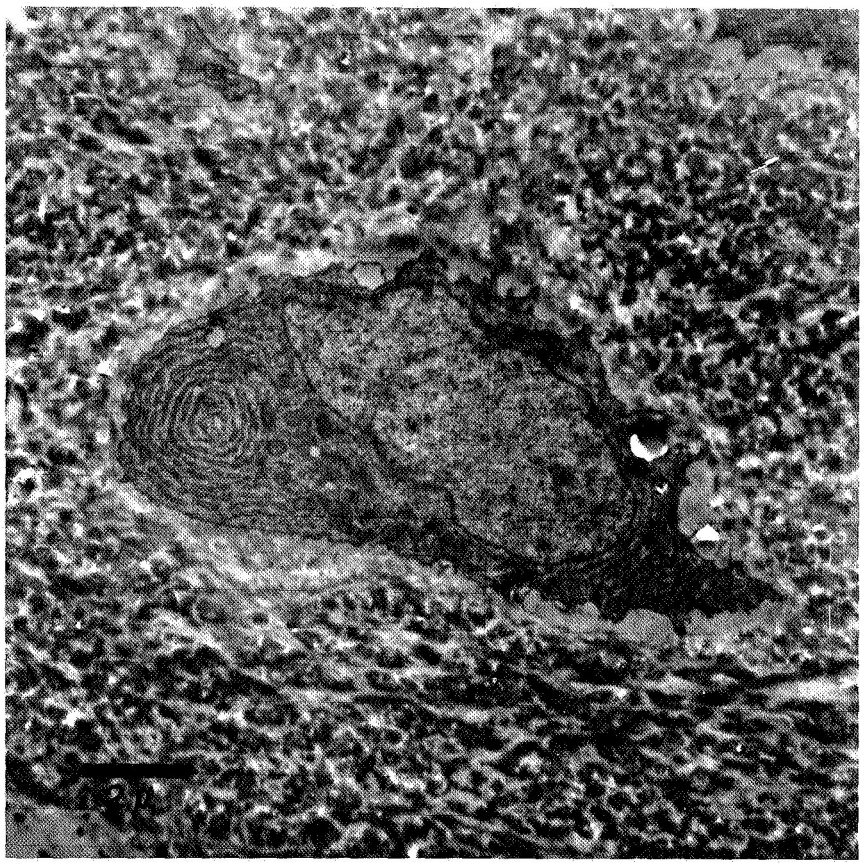

FIGURE 104. Peripheral osteocyte from the tibia of a 2-day-old chick embryo. The cell is closely encased by the matrix. Endoplasmic reticulum, ribosomes, and mitochondria are prominent features.

surface of the bone. They were osteocytes that were embedded in much larger lacunae in which the wall appeared to be rough.

In our own chick material (fig. 104) a young osteocyte is located near the surface. Actually, we can see that it fits quite neatly inside its lacuna and that it has most of its cytoplasm on one side of the nucleus, as an osteoblast might also have. We can see mostly endoplasmic reticulum, mitochondria, and some people can see a bit of Golgi complex even at this low power.

When we come to the osteocytes that are located deeper inside the trabeculae (fig. 105), we can now see large, osmiophilic vesicles. The size of the lacuna is also increased. Finally, the largest of these osteocytes, which we find in confluent lacunae in the center of the trabeculae, contain a larger number of these osmiophilic vesicles in their cytoplasm.

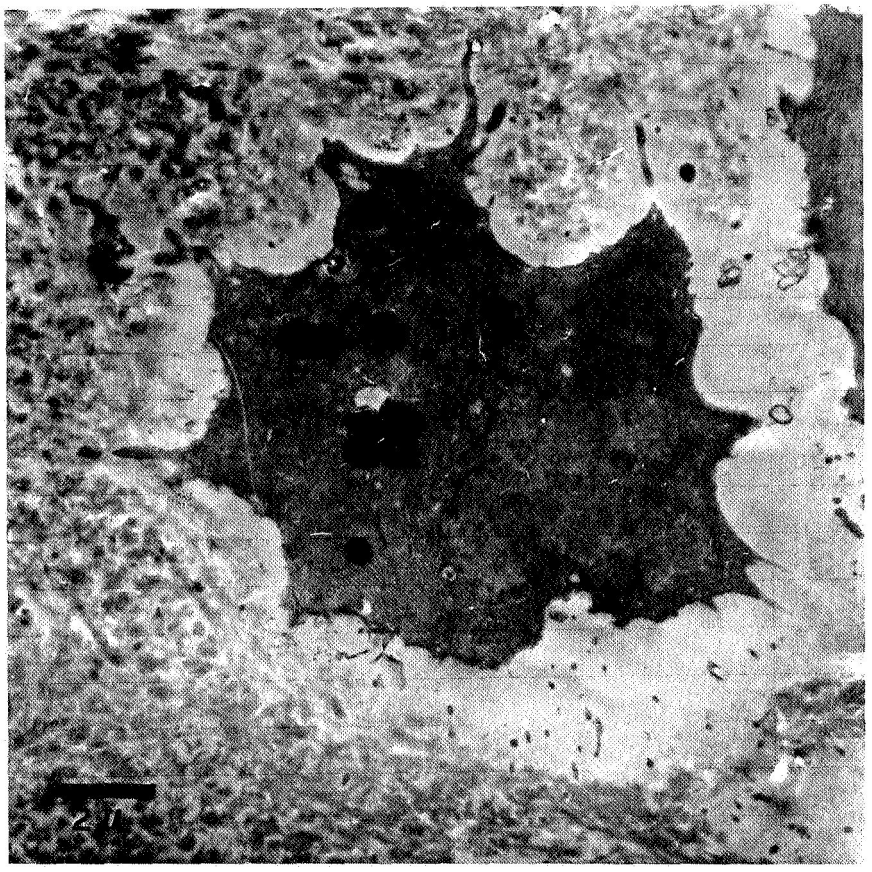

FIGURE 105. A centrally located osteocyte from the same specimen as in figure 104. The lacuna is wide and apparently confluent with an adjacent one. Vesicles containing osmiophilic material (lysosomes?) are prominent.

Some people say I should call them "lysosome-like" because I do not know whether or not they are lysosomes; they look like lysosomes.

If we give parathyroid hormone to some of these chick embryos, putting PTE in the yolk, 2 units per gram for a chick of 7 grams in weight, more or less, for 24 hours, this is what we see (fig. 106). The osteocytes near the surface apparently mature very rapidly. Now the endoplasmic reticulum has become enlarged, and there are cisternae, such as described by Baud (ref. 173). The osmiophilic vesicles which hardly exist in the young osteocyte become fairly numerous after parathyroid hormone, and we even begin to see a structure like this, which some electron microscopists tell me is an autophagic body. This apparently is related to cell digestion from enzymes which issue from some of the osmiophilic vesicles.

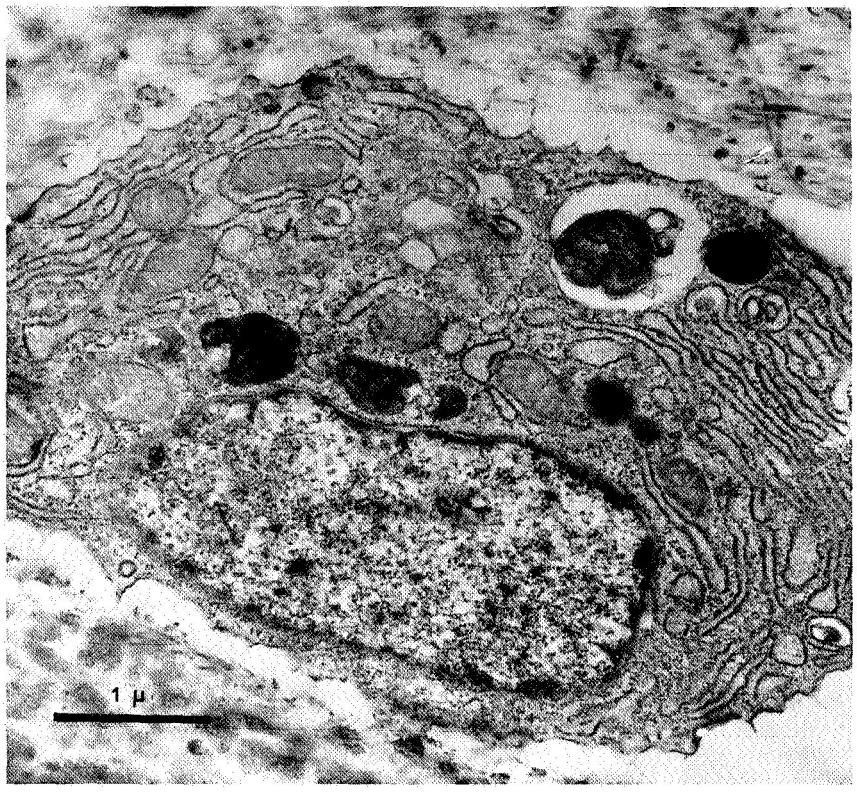

FIGURE 106. Peripheral osteocyte from an 11-day chick embryo treated with PTE. Enlarged cisternae and numerous pinocytic vesicles are visible; more numerous osmiophilic vesicles are also present; some appear to have been released into the lacuna.

So it is apparent that parathyroid hormone has increased the maturity of the cells, and has increased—as part of the maturity of the cells—the production of the osmiophilic vesicles which might be related to the production of protease. If this goes on for a time, the whole cell is destroyed, and we are left with an empty lacuna.

NICHOLS: I would like to say something about fluoride. I have one more figure about another osteoporotic patient.

We need to learn more about fluoride and its effects, because it is possible that we might learn how to treat osteoporosis even though we do not know what it is. Figure 107 shows roentgenographs of the lumbar spine and pelvis of a woman who came to me because she had developed acute pain in her back one day when she jammed on the brakes of her car. The picture on the left was made shortly after this episode when she was experiencing considerable pain from a crushed vertebra, and had obvious osteoporosis. One year later, she had the

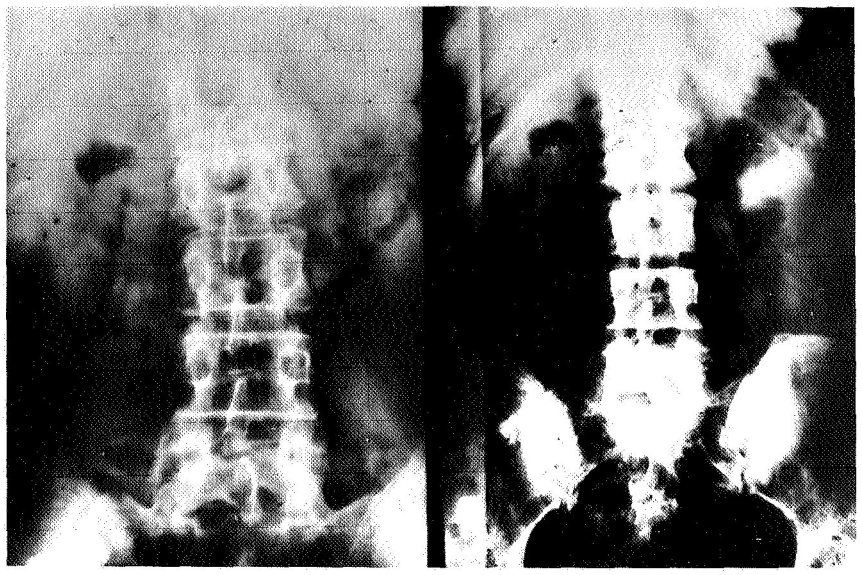

FIGURE 107. Roentgenographs of lumbar spine and pelvis of a 53-year-old female with osteoporosis (left) and after fluoride treatment (right).

picture on the right, which shows considerable thickening of the trabeculae in her vertebra and pelvis.

The woman was 53 years old. The clinical data which were found at that time are shown in the top half of table XXII. Serum calcium concentration was 4.6 mEq/l; alkaline phosphatase was normal, and urinary hydroxyproline excretion, which may or may not be an index of the rate at which collagen is destroyed in the body, was actually lower than normal of 28 milligrams for 24 hours. Metabolic studies of her bone biopsy showed normal lactate production and incorporation of glucose into collagen and cells. Proline incorporation into collagen was normal, but cell labeling was a little high and O_2 uptake was just above the normal range as we have defined it.

She was started at this point on 100 milligrams of sodium fluoride per day. About 5 months later she was completely symptom free and she has never had any pain since, nor does she have any evidence of any new crushed vertebra at this point. It took about 10 months for her to develop any roentgenographic change that we could recognize. When we did we biopsied her again.

This was 11 months after the first one. Serum calcium and so forth were normal. She did, however, show elevations of serum alkaline phosphatase activity and urinary hydroxyproline excretion. Bone metabolic data showed the same O_2 uptake but reduction in lactate production. The striking changes are in the measurements which re-

TABLE XXII

SKELETAL METABOLIC CHANGES IN IATROGENIC FLUOROSIS

[Female–Age 53]

Clinical data	July 1964 osteoporosis	June 1965 osteoporosis + fluorosis	Normal
Serum Ca, mEq/l	4.6	4.9	4.5–5.2
Serum F, mmoles/l	1.3	1.2	1.0–1.5
Alkaline phosphatase, B–L units	1.2	2.4	0.5–2.2
Urine hydroxyproline, mg/24 hr	15	40	24 ± 4.9
Bone metabolic data per mg DNA:			
Oxygen uptake, μmoles	0.92	0.91	0.47 ± 0.22
Lactate production, μmoles	1.17	0.72	0.79 ± 0.29
Glucose incorporation, mμ moles:			
Cells	551	901	320 ± 170.0
Collagen	4.60	20.7	4.00 ± 0.92
Proline incorporation, mμmoles:			
Cells	66.0	64.1	23.4 ± 7.84
Collagen	0.19	0.79	0.24 ± 0.09

late to matrix biosynthesis. Glucose incorporation is now three times the normal value. The ratio of glucose to collagen is five times normal and the incorporation of proline label into collagen and cells is similarly increased. In other words, these data show that the roentgenographic changes we call fluorosis are clearly reflected by increased bone-cell metabolism – especially in the area of bone collagen synthesis. This is important because it indicates that one can do something about this disease. One can substitute another disease for the one which is troubling the patient, a disease which is, relatively speaking, asymptomatic.

The question that we do not know the answer to as yet is, "What happens when fluoride is stopped?" This patient has been off fluoride now for 4 months. Her alkaline phosphatase is back down to the normal range, but whether this means that she is going to revert to her previous osteoporotic state I do not know. Time will have to supply the answer. She is, by the way, our one patient who has agreed to have a third biopsy.

HOWELL: Is that not a calcified paravertebral ligament on this patient's spine?

NICHOLS: Yes.

COPP: Is there any relationship between heparin-induced osteoporosis and the fluoride type, and vice versa?

NICHOLS: There appears to be, in fluorosis, a stimulation of the biosynthetic machinery to make more matrix. What we do not know is whether there is a simultaneous specific inhibition of the biosynthesis of collagenase or other catheptic enzymes. This remains to be looked into. Unfortunately, we do not have an analysis of collagenolytic activity prior to sodium fluoride in this woman.

The cause of the stimulation of matrix synthesis is not known either. In animals we have some observations which show that one can reproduce this biosynthetic stimulation. Dr. Peck showed data last spring indicating that collagen synthesis is inhibited by fluoride, and we have had the same experience at certain dose levels. On the other hand, if the animal lives long enough, the corner seems to be turned and a relative stimulation seems to begin. Dr. Peck, do you have a comment?

PECK: We were giving large doses of fluoride in drinking water to weanling rats, and the situations are obviously not quite comparable (ref. 153). Significant inhibition resulted, which was dose related. Our greatest effects occurred about 50 ppm for about 2 weeks to 1 month in a weanling rat. That is really a lot of fluoride.

URIST: Dr. Nichols, you have done a beautiful job. We will now adjourn.